ISFMS 2017

2nd International Symposium on Frontiers in Molecular Science

Non-Coding RNAs and Epigenetics in Cancer

21 – 23 June 2017, Biocenter, University of Basel

Program and Abstract Book

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Welcome by Prof. George A. Calin

Dear authors and attendees,
One of the most unexpected and fascinating discoveries in oncology over the past decade has been the interplay between abnormalities in protein-coding genes and non-coding RNAs (ncRNAs), which are causally involved in cancer initiation, progression, and dissemination. Although, to date, the most studied non-coding RNAs (ncRNAs) are miRNAs, the importance of long non-coding RNAs (lncRNAs) is increasingly being recognized. At the conference, entitled *"Non-Coding RNAs and Epigenetics in Cancer"*, leaders in the field will present the roles of miRNAs and lncRNAs in cancer, with a focus on the recently identified novel mechanisms of action, and discuss the current strategies in designing ncRNA-targeting therapeutics, as well as the associated challenges. We hope to see you all, young in spirit and mind, in the new Eldorado of Science topics in biomedical sciences!

The *Non-Coding RNAs and Epigenetics in Cancer* will be held in Basel, Switzerland, from 21st to 23rd of June 2017. It will comprise five plenary sessions to highlight the most exciting developments and the latest breakthroughs in oncology.

Prof. George A. Calin
Conference Chair

Welcome by Dr. Conradin Cramer

Welcome to Basel! We are proud to host such an eminent group of scientists in our city. On the other hand, we may modestly say, that this location is ideal for a high level symposium on molecular science in general and on cancer medication in particular.

Basel is a hot spot for Life Sciences. The Life Sciences form the basis of our economy and are an important source of our public wealth. Moreover Basel has an all-embracing scientific ecosystem in the Life Sciences. The two global players Novartis and Roche alone contribute a volume of about 6 billion Swiss francs from Life Science Research. Although financed at a much lower level our University has a strong focus and hits well above its size in the Life Sciences – a knowledge pipeline beginning with the basic research at the Biozentrum and continuing along a translational path to the clinical research and practical application in the University Hospitals. The University is associated with renowned research institutes such as the Friedrich Miescher Institute of Novartis and the Swiss Tropical and Public Health Institute (Swiss TPH), which, like the University is a public institution. Of course the University also cooperates with top Universities in Switzerland – especially the ETH Zurich – and worldwide. There is also a close cooperation with our regional University of Applied Sciences.

If you look out of the window you will see the Life Science Campus arising, on which you soon will find the ETH-Department of Bio Systems and Systems Engineering in close vicinity to our univer-sity-institutes, such as the departments of Chemistry and Physics, the Biozentrum and the De-partment of Biomedical Science. In the same area we find the campus of our University hospital. We thus combine all of the essential components for a successful translation from high end re-search to clinical application in an area less than a square kilometre.

How can this be done by a little administrative region such as Basel City with only 190‘000 inhab-itants? It is possible because of strong regional partnerships, especially with our neighboring county, that sends the greatest cohort of students to the University of Basel. Our entire region is proud of its university and participates to its wellbeing, financially, culturally and emotionally. And of course there are the subsidies of the federal government as well as national and international research grants which are acquired with great success by our university and provide the fuel for the research engine. The whole ecosystem is embedded in a trinational region, where the three nations France, Germany and Switzerland meet and form one of the strongest Life-Science clus-ters of the world. A region of frontiers, ideally prepared to test and move beyond the frontiers in molecular sciences!

In Basel you encounter a curious combination of small town with a strong identity and a worldwide scientific impact. We believe this to be a unique strength of this area. The short distance between centres of excellence facilitates free exchange between scientists and leads to an openness to other cultures and a profound commitment to the improvement of life quality worldwide. Your meeting here is a fine signal of this ambition.

With this brief welcome note I hope to have shown, that you have chosen excellent surroundings for your congress. I wish you inspiration, a good atmosphere and of course scientific success. However, please don't forget to explore our other highlights: the fine architecture of Basel, the cultural highlights and, of course, the culinary opportunities. In short: I hope you will also find a little time for leisure and enjoyment during your stay here.

Dr. Conradin Cramer
Governing Counsellor Canton Basel-Stadt
Head of the Department of Education

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Message from the Editor-in-Chief

The *International Journal of Molecular Sciences* (*IJMS*, ISSN1422-0067) is an open access journal, which was established in 2000. The journal aims to provide a forum for scholarly research on a range of topics, including biochemistry, molecular biology, pathology and toxicology, chemistry, material science and molecular physics (chemical physics and physical chemistry). *IJMS* publishes both original research and review articles, and regularly publishes special issues to highlight advances at the cutting edge of research. We invite you to read recent articles published in *IJMS* and consider publishing your next paper with us.

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- Bioactives and Nutraceuticals
- Translational Medicine
- Bioinorganic Chemistry
- Molecular Botany

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Basel, March 2017

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Editor-in-Chief

Prof. Dr. George A. Calin

Message from the Editor-in-Chief

This field finally has a dedicated journal where its broad community can communicate and exchange its latest findings in one centralized place. This field was built stone by stone from the many scientific contributions from extremely diverse horizons, studying gene silencing in plants, position effect variegation in drosophila or quelling in fungi. This field has achieved maturity, but a lot remains to be discovered! Our aim is to publish manuscripts from all horizons that will have a high impact on the development of the field. Let's have fun and wish *Non-Coding RNA* a long and rewarding life!

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- **Free publication in 2017** The publication fee will be fully waived for papers submitted in 2017

Aims and Scope

Non-Coding RNA (ISSN 2311-553X) is an open access journal which provides an advanced forum for research studies on non-coding RNAs and their regulatory roles. We encourage the publication of original research papers, short reports, communications, snapshots and conference reports, timely reviews and of commentaries on hot topics of interest to the non-coding RNAs community.

The scope of *Non-Coding RNA* includes, but is not limited to, the following subjects:

- Functional studies dealing with identification, structure-function relationships or biological activity of all types of non-coding RNAs
- Analysis of RNA processing, RNA binding proteins, RNA signaling and RNA interaction pathways
- RNA analyses, bioinformatics, new tools and technologies
- Translational studies involving long and short non-coding RNAs

Non-Coding RNA regularly publishes special issues on relevant and timely topics. Check our website for an updated list of special issues.

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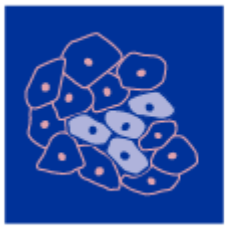
Basel, May 2017

1 General Information



cancers

Cancers (ISSN 2072-6694) is an international, peer-reviewed open access journal on oncology. It publishes reviews, regular research papers and short communications. Our aim is to encourage scientists to publish their experimental and theoretical results in as much detail as possible. There is no restriction on the length of the papers. The full experimental details must be provided so that the results can be reproduced. There are, in addition, unique features of this journal:

- manuscripts regarding research proposal and research ideas will be particularly welcomed
- electronic files or software regarding the full details of the calculation and experimental procedure, if unable to be published in a normal way, can be deposited as supplementary material
- we also accept manuscripts communicating to a broader audience with regard to research projects financed with public funds
- we accept studies showing meaningful but negative results.

While there are many journals that focus on cancer studies, none of them actively accepts negative results. As a result, most negative data end up not being in the public domain even if the data were meaningfully negative and the study well designed. By accepting those negative results, our journal encourages scientists to share those data so that they would not need to repeat the experiments that somebody else has already done.

Tracked for **Impact Factor**
Citescore: 5.02 Scopus

www.mdpi.com/journal/cancers

The ***Non-Coding RNAs and Epigenetics in Cancer*** will be held in Basel, Switzerland, from 21st to 23rd of June 2017. It will comprise five plenary sessions to highlight the most exciting developments and the latest breakthroughs in oncology.

1.1 Conference Topics

- The Biology of ncRNAs
- Translational Applications of ncRNAs as Biomarkers
- Bioinformatics and ncRNAs World
- ncRNA Therapeutics
- ncRNA Technologies

1.2 Conference Venue

Biocenter/PharmaCenter
Universität Basel
Klingelbergstrasse 50, CH-4056 Basel, Switzerland

1.3 Registration Desk

21 June –23 June 2017
07:30–17:30
Direct Telephone Line: +41 61 267 20 06

1.4 Wireless Internet Access

WLAN: unibas-event
Login: bzpzevent
Password: ISFMS-2017

1.5 Directions and Map

1.6 Switzerland and the Tri-National-Region

Basel lies in the heart of Europe, on both banks of the Rhine. The city is the center of the idyllic border triangle of France, Germany and Switzerland—lying between the Swiss Jura, Germany's Black Forest and the Vosges in Alsace.

Basel is so easy to get to. Only a 10-minute drive from the city center, Basel's EuroAirport is served by a number of international airlines. Together with neighbouring Zürich Airport, it enjoys connections to all European airports and to more than 200 intercontinental destinations.

Located in the center of Europe, Basel is a major transportation hub. Its three railway stations not only offer excellent connections to far and wide but are also all situated in the very heart of the city. [Source: www.basel.com]

1.7 Basel

Where the Rhine, one of Europe's most important waterways, bends north and flows out of Switzerland towards the North Sea lies the charming city of Basel. This exceptional location at the heart of the three-country-triangle that joins Germany, France and Switzerland is what lends Basel its openness, economic strength and cultural diversity. [Source: www.bs.ch]

1.8 Best Connections

As far back as the Middle Ages, Basel became a major transportation hub thanks to its location on the Rhine and in the center of Europe. And still today, there is no way around Basel: The city lies at the intersection of the German and French rail and road networks. The trinational EuroAirport Basel-Mulhouse-Freiburg and the Rhine port connect Basel with the world. [Source: www.bs.ch]

1.9 Dynamic Economy

Again thanks to the Rhine, Basel developed into a prosperous center for commerce and trade fairs early on. Today, this city with a total area of only 37 square kilometres, inhabited by 200,000 people from 160 countries, is at the heart of the most dynamic economic region in Switzerland. [Source: www.bs.ch]

1.10 Fair Weather City

Next to the rich cultural offerings (museums with a global reputation, theater and concert halls, renowned architecture), the weather adds to the high quality of life: Nestled comfortably in the Rhine valley, Basel enjoys many more days of sunshine than the towns in central Switzerland. [Source: www.bs.ch]

1.11 The University of Basel

The University of Basel has an international reputation of outstanding achievements in research and teaching. Founded in 1460, the University of Basel is the oldest university in Switzerland and has a history of success going back over 550 years.
As a comprehensive university offering a wide range of high-quality educational opportunities, the University of Basel attracts students from Switzerland and the entire world, offering them outstanding studying conditions as they work towards their bachelor's, master's or PhD degrees. Today, the University of Basel has around 13,000 students from over a hundred nations, including 2,700 PhD students. The University of Basel has seven faculties covering a wide spectrum of academic disciplines. At the same time, the university has positioned itself amidst the international competition in the form of five strategic focal areas: Life Sciences, Visual Studies, Nanosciences, Sustainability and Energy Research and European and Global Studies. In international rankings, the University of Basel is regularly placed among the 100 top universities in the world thanks to its research achievements. [Source: www.unibas.ch]

1.12 Biocenter/PharmaCenter

The Biozentrum (Biocenter), is the largest department at the University of Basel's Faculty of Science. The primary focus of this interdisciplinary institute is basic molecular and biomedical research and teaching. The Biozentrum holds a leading position nationally and internationally and closely networks with partners from the academic world and industry.

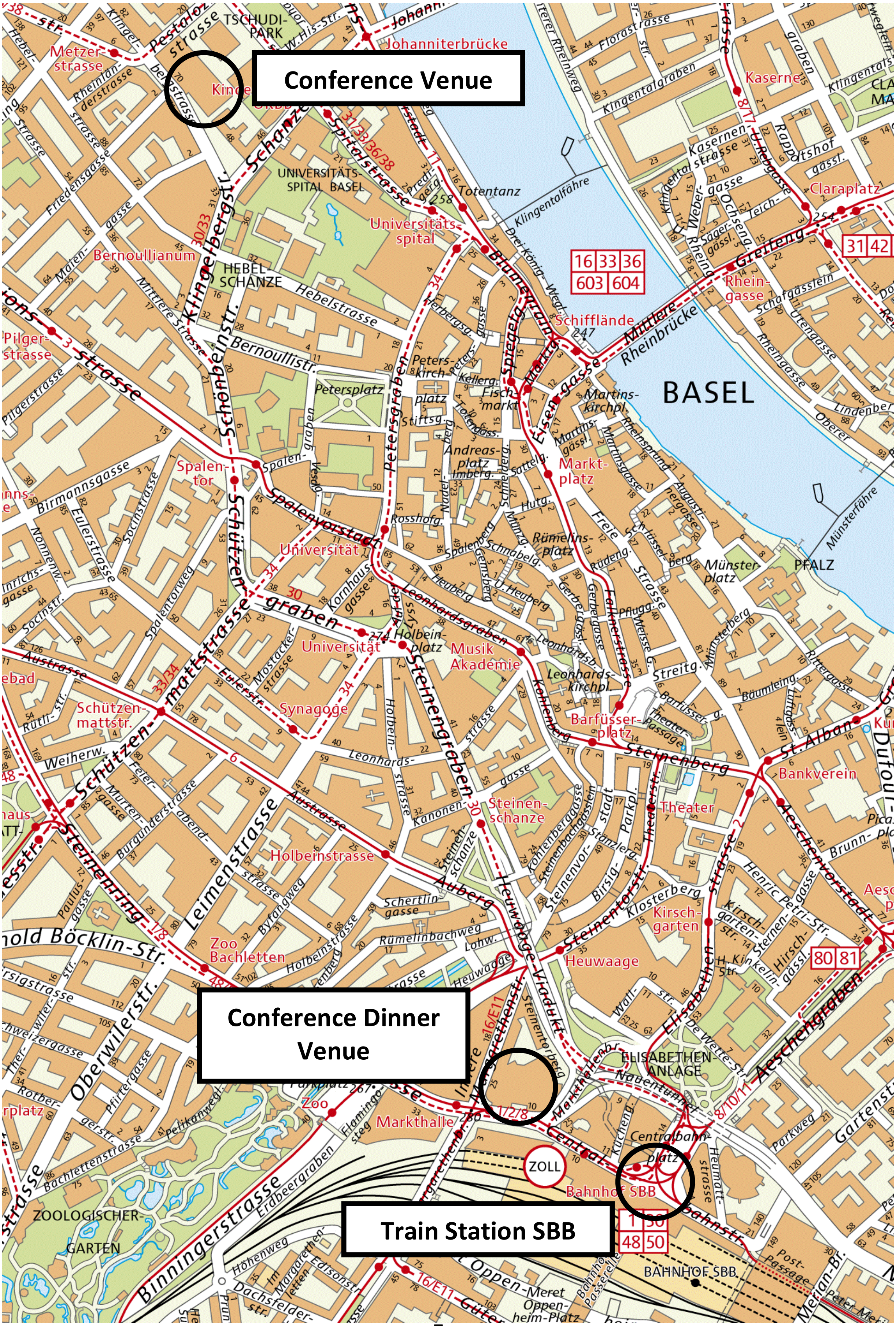
In 1971, at the time when the Biozentrum was founded, the visionary concept of developing an interdisciplinary research facility was unique. Today, some 40 years later, the success of this interdisciplinary approach to molecular and biomedical research remains evident. It continues to be the Biozentrum's greatest strength, along with its excellent facilities providing leading technologies and its highly motivated staff.

The Biozentrum is home to 30 research groups. These scientists, representing more than 40 nations, are engaged in investigating the molecular basis of biological processes. Their work covers a broad spectrum of activities, the scientific research is wide-ranging: How does a cell develop, how does it function and how are all its vital processes regulated? Can we make computer assisted models of these processes? How does a stem cell know what to become? How does a blood vessel form or the nervous system develop and how does the body defend itself against bacterial infections? Could the findings lead to new approaches in the treatment of serious diseases such as muscular diseases, Alzheimer's disease or cancer? Producing more than 200 scientific publications each year, the Biozentrum is regularly rated in the top 25% of the world rankings. Research at the Biozentrum is grouped into five major focal areas: Growth and Development, Infection Biology, Neurobiology, Structural Biology and Biophysics, as well as Computational and Systems Biology. These research areas, however, are not strictly separated from each other; new and relevant questions often arise at the overlap between the research fields, while the collaboration between teams and the expertise of each respective area leads to innovative solutions. This has contributed greatly to the scientific success of the Biozentrum. Both its funding and infrastructure make the Biozentrum internationally highly competitive and ensures research of the highest level.

The Biozentrum enjoys an excellent reputation for its scientific training, both nationally and internationally. Students are integrated into a research environment from the start of their academic career and gain first-hand experience of life as a scientist. Being able to link education with research makes the Bachelor's and Master's degree programs at the Biozentrum particularly attractive for many aspiring students. PhDs and postdocs, on the other hand, benefit from the Biozentrum's scientific success and the intensive, individual supervision.

The PharmaCenter Basel, The University of Basel Translational Science Platform, is the interdisciplinary center for excellence at the University of Basel. The PharmaCenter Basel aims to establish a leading research and teaching community in drug development, drug therapy and drug safety. Together with partners from the industry, the PharmaCenter Basel plans to translate increased knowledge about the molecular basis of disease into improved therapies. [Sources: www.biozentrum.unibas.ch and https://pharmacenter.unibas.ch/]

1.13 Location



1.14 How to Reach the Venue

Public Transport

From **EuroAirport Basel Mulhouse Freiburg** (15 minute journey)
Take the airport bus (No. 50) to the Kannenfeldplatz stop, where you have to change onto a No. 31, 36 or 38 bus going in the direction of Schifflände/Habermatten or Wyhlen Siedlung. Get off at the next stop, Metzerstrasse, and cross the road to the Biocenter/PharmaCenter.

From the **Basel SBB** (Swiss) and SNCF (French) train station (15 minute journey)
Take a No. 30 bus to the Kinderspital UKBB (children's hospital) stop and cross the road to the Biocenter/PharmaCenter–see Google Maps.

From the **Badischer Bahnhof** (German) train station: (10 minute journey)
Take a No. 30 bus to the Kinderspital UKBB (children's hospital) stop, and then walk to the Biocenter/PharmaCenter–see Google Maps.

By Car

Within Switzerland
Leave the motorway in the direction of the Unispital, drive through the tunnel and then across the viaduct. Keep on the main road, passing Spalentor, and carry straight on over the traffic lights. Turn left after about 500 m. The Biocenter/PharmaCenter is then on the right-hand side.

From France
After driving over the border in Saint-Louis, drive towards Basel-Kannenfeld as indicated. Stay on the main road (direction city), go straight on around the roundabout (direction city) and, after about 500 m, take the left-hand lane at Kannenfeldplatz. After only a few meters, take the right-hand lane and turn into Metzerstrasse. The Biocenter/PharmaCenter is then about 300 m ahead.

From Germany
Leave the expressway at exit Basel-St. Johann. After the tunnel, carry straight on for about 150 m. Turn left into Elsässerstrasse (direction city) and then, after 550 m, right onto St. Johanns-Ring (direction Augenspital). After 300 m, turn left into Klingelbergstrasse. The Biocenter/PharmaCenter is then on the left-hand side.
[Source: www.biozentrum.unibas.ch]

1.15 Inside the Biocenter/PharmaCenter

1.16 Conference Dinner

Thursday, 22 June 2017, 19:00

Old Market Hall, Basel

The conference dinner will take place at the Old Market Hall next to Basel SBB. Dating from 1929, the landmark building with its magnificent cupola (the third largest of its type in the world) has been a symbol of the city for over 80 years. The inviting aromas of fresh bread, sweet fruits and freshly ground coffee can be experienced under the large cupola of the Old Market Hall. The attractive domed building has been in use as a market hall again since October 2013, featuring market stalls well-stocked with fresh products and appetising menus. Comfortable seating and free WLAN mean that eating, drinking and passing the time here is a unique opportunity. [Sources: www.bs.ch and www.fohhn.com]

Markthalle Basel
Steinentorberg 20
4051 Basel

www.altemarkthalle.ch

1.17 Visiting Basel and Dining Out

It is not easy to describe Basel in a few words. Descriptions for example such as the “cultural city of Switzerland” or “University town” are merely an attempt to give some sort of impression of the wealth of culture, history, relaxation and enjoyment to be found in the city. Whether it is a visit to one of the numerous museums, a dip in the Rhine or an evening at the theatre, allow yourself to be inspired by the joys that await you in Basel.

Art and Culture

Fondation Beyeler—www.fondationbeyeler.ch

In building Renzo Piano’s museum in 1997, the Fondation Beyeler made its collection accessible to the public. The 250-odd works of classic modernism reflect the views of Hildy and Ernst Beyeler on 20th-century art and highlight features typical of the period: from Monet, Cézanne and van Gogh to Picasso, Warhol, Lichtenstein and Bacon. The paintings appear alongside tribal art from Africa, Oceania and Alaska.

Museum Tinguely—www.tinguely.ch

Situated directly on the Rhine, the Museum Tinguely, built according to plans by the Ticinese architect Mario Botta, houses the greatest collection of works by Jean Tinguely (1925–1991), one of the most innovative and important Swiss artists of the 20th century. The permanent exhibition presents a survey of his oeuvre spanning four decades. Special exhibitions show a wide range of artists and subjects including Marcel Duchamp and Kurt Schwitters who influenced Tinguely significantly, companions such as Arman, Niki de Saint Phalle, Yves Klein as well as current art trends along Tinguely’s ideas.

Vitra Design Museum—www.design-museum.de

The Vitra Design Museum numbers among the world’s most prominent museums of design. It is dedicated to the research and presentation of design, past and present, and examines its relationship to architecture, art and everyday culture. In the main museum building by Frank Gehry, the museum annually mounts two major temporary exhibitions. In conjunction with our alternating exhibitions, the Vitra Design Museum offers a variety of workshops and guided tours. [Source: www.basel.com/en]

Suggestions of Restaurants in Basel—www.basel.com

***Kohlmanns*—www.kohlmanns.ch**

It smells of fire, wood and freshly baked foods. The restaurant with its modern oak furniture is extremely cosy and is situated right at the Barfüsserplatz. *Kohlmanns* offers hearty Swiss and surprising regional specialities.

***Brasserie au Violon*—www.au-violon.com**

Lively brasserie with traditional and seasonal French cuisine served in a former prison.

***Zum Braunen Mutz*—www.brauner-mutz-basel.ch**

The traditional tavern with bar and restaurant. Here you will meet original Basel locals of all generations.

***Der vierte König*—www.weinwirtschaft.ch**

In the restaurant *Der vierte König* you will find freshly cooked meals and a fine selection of bottled wines from all over the world.

***Kunsthalle*—www.restaurant-kunsthalle.ch**

The traditional restaurant *Kunsthalle*, where "Tout Bâle" feels at home serves seasonal delicacies.

***Brasserie Monsieur Verseau*—www.brasserie-verseau.ch**

This brasserie at Messe Basel (convention centre) impresses with its casual, cosy atmosphere combined with modern architecture. The menu features hearty regional dishes and French classics.

***Cheval Blanc*—www.lestroisrois.com**

Refined seasonal cuisine and a selected wine list. Awarded with 19 points Gault-Millau and two Michelin stars. Summer terrace with a great view of the Rhine.

***Chez Donati*—www.lestroisrois.com**

For more than 50 years, the *Chez Donati* is an esteemed institution and the essence of fine Italian table culture in Basel.

***Brasserie Les Trois Rois*—www.lestroisrois.com**

The relaxed atmosphere and Swiss and French brasserie specialities make the city restaurant in the *Les Trois Rois*, a 5-star-superior deluxe hotel, a popular all-day rendezvous.

***Atelier* (*Der Teufelhof*)—www.teufelhof.com**

The restaurant charms by its modern and inspiring ambience. Enjoy a modern international cuisine with predominatly Swiss and regional products.

***Les Quatre Saisons*—www.lesquatresaisons.ch**

Treat yourself to some culinary delights in the newly renovated Restaurant *Les Quatre Saisons.* Head chef Peter Moser and his team apply a fresh sense of inspiration and a high level of commitment to their dishes, bringing together all of the elements necessary to create their unique cuisine–ingredients fresh from the market, original recipes and a great deal of passion.

Suggested Events

Malevich, Kandinsky and revolutionary porcelain — Russian masterpieces of art and *white gold* from 1917 to 1927 — www.spielzeug-welten-museum-basel.ch/en/

Russian porcelain of the period from 1917 to 1927 reflects the dramatic changes in Russian life at the time. Wholly unique, thematically contemporary decorations are typical. Having emerged in the atmosphere of the Russian Revolution, this white gold of the 1920s was used for more than just propaganda and didactic purposes. In a period dominated by industrial design, many outstanding artists turned to this as the art form most likely to reach the broad masses. Technically superb craftsmen modelled their creations after designs by the artists. This combination yielded amazingly beautiful, never-before-seen porcelain pieces that were often only made as one-offs or in small series.

In this unique special exhibition, over 300 select porcelain pieces from the finest private collection are displayed publicly for the very first time. On display are creations from 64 avant-garde artists such as Kazimir Malevich, Vasily Kandinsky, and Nikolai Suetin, to name some of the most prominent ones. The exhibit is rounded out with drawings and designs from these renowned masters as well as David Yakerson.

Let yourself be inspired by a breathtakingly creative era of Russia’s history, with something new and surprising at every turn.

Elytra Filament Pavilion — Outdoor installation in the context of the exhibition »Hello, Robot.« — www.design-museum.de

With the exhibition »Hello, Robot. Design between Human and Machine«, the Vitra Design Museum presents a major exhibition that examines the current boom in robotics. It shows the variety of forms that robotics takes today and at the same time broadens our awareness of the associated ethical, social, and political issues. Outside the museum, the »Elytra Filament Pavilion« complements this exhibition. The bionic baldachin is an impressive example of the growing influence of robotics on architecture. Its individual modules were defined by an algorithm and then produced with the help of an industrial robot, realised by a team from the University of Stuttgart. After its premiere at the Victoria & Albert Museum in London, it is now on view on the Vitra Campus.

¡HOLA PRADO! — Two collections in dialogue — kunstmuseumbasel.ch/en/

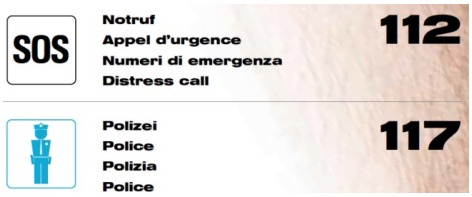
A return visit between friends and a generous gesture by one of the world's most significant art collections: In the summer of 2015 the Kunstmuseum Basel lent ten paintings by Pablo Picasso to the Museo Nacional del Prado in Madrid, where they were seen by around 1.4 million visitors. This year, the Prado is repaying the favour by sending twenty-six master works dating from between the late fifteenth century and the close of the eighteenth century on a journey to Basel.

Even such a generous return loan cannot hope to reflect the full richness of the Madrid collection. Therefore, the selection agreed by the Kunstmuseum and the Prado deliberately eschews the attempt to show a cross-section of our respective holdings. Instead, the handpicked guests from the Prado are shown in a sequence of twenty-four focused encounters with a corresponding selection of works from the Kunstmuseum: Titian, Zurbarán, Velázquez, Murillo and Goya appear in dialogue with Memling, Baldung, Holbein the Younger, Goltzius and Rembrandt. Prints by Goya and Holbein the Younger, from the holdings of the Department of Prints and Drawings in Basel, conclude the summit meeting between the two museums. The aim of the exhibition is to identify and make visible the points of connection, bridging artistic, geographical and historical divides, between pictures and collections. A journey of discovery, replete with artistic pleasures, awaits the visitor.

RICHARD SERRA — Flms and videotapes — kunstmuseumbasel.ch/en/

Richard Serra (b. San Francisco, 1938) is one of the most influential artists working today. He is best known for the monumental steel outdoor sculptures he has made since the 1970s; several of these have sparked public controversies—including in Basel, where his sculpture Intersection on Theaterplatz is a striking sight in the urban fabric. The structures and sculptures Serra creates elicit complex aesthetic experiences that speak forcefully to our relationship with our surroundings—be it the built urban environment or a landscape—and our perception of the world.
The exhibition Richard Serra: Films and Videotapes at the Kunstmuseum Basel | Gegenwart turns the spotlight on Serra's work in film, which goes back to 1968 and has been a crucial source of impulses for his artistic and experimental use of both media. The artist also recruited several people to work with him on his films who went on to distinguished careers in the fields of visual art and filmmaking, including Joan Jonas, Nancy Holt, and Babette Mangolte.
The show presents sixteen films and videos Richard Serra made between 1968 and 1979. All works will be screened in the original formats. Richard Serra: Films and Videotapes is the first exhibition to offer such a comprehensive survey of Serra's entire output on film: although art theorists regard these works as a vital component of his oeuvre, they have rarely been shown as a cohesive ensemble—perhaps in part because it is difficult to screen 16 mm footage in constant quality over an extended period of time. We at the Kunstmuseum Basel believe it is important to provide a stage to the moving image in Serra's oeuvre.

1.18 Emergency Information



Other useful numbers

Medical Emergency Center	+41 (0) 61 261 15 15
REGA air rescue service	1414

microarrays

Microarrays (ISSN 2076-3905), is an academic Open Access Journal for microarray technology and applications. It publishes original research papers, comprehensive reviews and communications. Our aim is to encourage scientists to publish their experimental and theoretical results in as much detail as possible. There is no restriction on the length of the papers. The full experimental details must be provided so that the results can be reproduced.

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2 Conference Program

2.1 Program at a Glance

	Wednesday, 21 June 2017	Thursday, 22 June 2017	Friday, 23 June 2017
Morning	**The Biology of ncRNAs** Prof. Reuven Agami Prof. Amy Pasquinelli	**Bioinformatics and ncRNAs World** Prof. Isidore Rigoutsos Prof. Mihaela Zavolan	**ncRNA Technologies** Prof. Thomas Schmittgen Prof. Jo Vandesompele
	Coffee Break	Coffee Break	Coffee Break
	The Biology of ncRNAs Prof. Qihong Huang Prof. Sven Diederichs	**Bioinformatics and ncRNAs World** Prof. John S. Mattick Prof. Ulf Andersson Orom Prof. Manel Esteller	**ncRNA Technologies** Prof. Maite Huarte Martinez
	Lunch & Poster Session	Lunch & Poster Session	Concluding Remarks
Afternoon	**Translational Applications of ncRNAs as Biomarkers** Prof. Carlo Croce Prof. Menashe Bar-Eli	**ncRNA Therapeutics** Prof. Frank J. Slack Prof. George A. Calin	
	Coffee Break	Coffee Break	
	Translational Applications of ncRNAs as Biomarkers Prof. Helge Grosshans Prof. Fabbri Muller Prof. Manuela Ferracin	**ncRNA Therapeutics** Prof. Massimo Negrini Prof. Kalpana Ghoshal	
		Conference Dinner	

2.2. Detailed Program

Day 1: Wednesday 21 June 2017	
07:30–08:30	Check-in
08:30–08:45	Welcome – Conradin Cramer
08:45–09:00	Introduction – George A. Calin
09:00–13:20	**Session 1: The Biology of ncRNAs – Sponsored by Arraystar**
Session Chairs:	**Prof. Amy Pasquinelli and Prof. Reuven Agami**
09:00–09:30	Reuven Agami – Functional Genetic Screens of Regulatory DNA Elements
09:30–10:00	Amy Pasquinelli – Elucidating the Dark Side of the MicroRNA
10:00–10:20	Joost Kluiver – ZDHHC11 and ZDHHC11B are Novel Critical Components of the Oncogenic miR-150-MYBnetwork in Burkitt Lymphoma
10:20–10:40	Eleonora Candi – Ultra-Conserved Non-Coding Transcript T-UC291 Controls Somatic Tissue Differentiation by Interfering with ACTL6A
10:40–11:00	Jelena Kresoja-Rakic – lncRNA RP11-334E6.12 expression is highly correlated with increased THY-1 expression in chemoresistant primary mesothelioma cells
11:00–11:40	**Coffee Break**
11:40–12:10	Qihong Huang – Lost in Translation: Long Non-coding RNAs in Metastasis
12:10–12:40	Sven Diederichs – MALAT1 and Beyond - Long Non-coding RNAs in Lung Cancer
12:40–13:00	Daniela Zeitler – Hyper-phosphorylation of Argonaute Proteins Affects mRNA Binding and is Essential for microRNA-guided Gene Silencing
13:00–13:20	Carmen Jeronimo – A Multiplatform Approach Identifies miR-152 as a Novel Epigenetically Downregulated microRNA in Prostate Cancer
13:20–14:30	**Lunch and Poster Session**
14:30–18:20	**Session 2: Translational Applications of ncRNAs as Biomarkers**
Session Chairs:	**Prof. Carlo Croce and Prof. Menashe Bar-Eli**
14:30–15:00	Carlo Croce – MicroRNA Dysregulation to Identify Therapeutic Target Combinations for Chronic Lymphocytic Leukemia
15:00–15:30	Menashe Bar-Eli – RNA Editing and Melanoma Metastasis
15:30–15:50	Stefan Eichmüller – miRNAs Modulating Melanoma Cell Invasion
15:50–16:30	**Coffee Break**
16:30–17:00	Helge Grosshans – Noncoding RNA Function and Regulation in Animal Development
17:00–17:30	Fabbri Muller – Role of Exosomal miRNAs in the Biology of the Tumor Microenvironment
17:30–18:00	Manuela Ferracin – Epigenetic Biomarkers of Prognosis in Stage IIA Colon Cancer
18:00–18:20	Dror Avni – Alterations of MicroRNAs Throughout the Malignant Evolution of Cutaneous Squamous Cell Carcinoma: The Role of miR-497 in Epithelial to Mesenchymal Transition of Keratinocytes

Day 2: Thursday 22 June 2017	
09:00–13:10	**Session 3: Bioinformatics and ncRNAs World**
Session Chairs:	**Prof. Isidore Rigoutsos and Prof. John S. Mattick**
09:00–09:30	Isidore Rigoutsos – Transcriptomic Heterogeneity: Known and Novel Short Non-coding Regulatory RNAs that Depend on Sex, Population Origin, Tissue, and Disease
09:30–10:00	Mihaela Zavolan – The 3' UTR Landscape of Human Cancers
10:00–10:20	Giovanna Brancati – Fatal Imperfections: Determinants of miRNA Target Specificity
10:20–10:40	Francisco J. Enguita – miRNAtools: Advanced Training Using the miRNA Web of Knowledge
10:40–11:00	Rory Johnson – Cancer Driver Long Noncoding RNA Discovery in the Pan-Cancer Analysis of Whole Genomes (PCAWG) Collaboration
11:00–11:40	**Coffee Break**
11:40–12:10	John S. Mattick – Exons are the modular unit of structure-function in regulatory RNAs
12:10–12:40	Ulf Andersson Orom – Chromatin-Release Is Important for Long NcRNA Function
12:40–13:10	Manel Esteller – Epigenetics and Epitranscriptomics of Non-Coding RNAs in Human Cancer
13:10–14:10	**Lunch and Poster Session**
14:10–17:50	**Session 4: ncRNA Therapeutics**
Session Chairs:	**Prof. Frank J. Slack and Prof. Massimo Negrini**
14:10–14:40	Frank J. Slack – MicroRNA-Based Therapeutics in Cancer
14:40–15:10	George A. Calin – About Chomsky, Non-Coding RNA Structure and Cancer Patient's Treatment
15:10–15:30	Aniello Russo – Role of miR-125a in Hepatic Carcinogenesis
15:30–16:10	**Coffee Break**
16:10–16:40	Massimo Negrini – Multiple Approaches for miRNA-based Therapies of Cancer
16:40–17:10	Kalpana Ghoshal – Transcriptome-Wide Mapping of the miR-122 Targetome Revealed its Mechanistic Role in the Maintenance of Liver Homeostasis and Suppressing Hepatocarcnogenesis
17:10–17:30	Silvia Catuogno – Development of RNA Aptamers for Targeting B-cell-derived Malignancies
17:30–17:50	Alva Rani James – A Specific long non-coding RNA Expression Signature Defines the Philadelphia-like B-cell Acute Lymphoblastic Leukemia Subtype
19:00	**Conference Dinner**

Day 3: Friday 23 June 2017	
09:00–13:20	**Session 5: ncRNA Technologies**
Session Chairs:	**Prof. Thomas Schmittgen and Prof. Manel Esteller**
09:00–09:30	Thomas Schmittgen – microRNAs Shape Plasticity of Pancreatic Acini
09:30–10:00	Jo Vandesompele – Tools for lncRNA Research in Cancer
10:00–10:20	Paola Parrella – Stepwise Analysis of MIR9 Loci Identifies miR-9-5p to be Involved in Oestrogen Regulated Pathways in Breast Cancer Patients
10:20–10:40	Jörg Krummheuer – An Improved Algorithm for Antisense LNA™ GapmeR Design
10:40–11:00	Mattia Boeri – High Risk Plasma microRNA Signature is Associated with an Immune-related Gene Expression Profile of Lung Tumour Tissues
11:00–11:40	**Coffee Break**
11:40–12:00	Martin Pichler – MicroRNA-196 Influence Metastases Formation in Colorectal Cancer through Regulation of HOXB and GALNT Gene Expression
12:00–12:30	Maite Huarte Martinez – Functional long Noncoding RNAs in Cancer Pathways
12:30–12.50	Enrique Fuentes-Mattei – Plasma Viral miRNAs as Targeted-biomarkers of Occult Viral Infections Prevalence and Sepsis Aggressiveness
12:50–13:10	Britta Skawran – The microRNA-449 Family Inhibits TGF-β-mediated Liver Cancer Cell Migration by Targeting SOX4
13:10	**Concluding Remarks**

3
Oral Presentation Abstracts

antibodies

Antibodies (ISSN 2073-4468), an international, peer-reviewed open access journal which provides an advanced forum for studies related to antibodies and antigens. It publishes reviews, research articles, communications and short notes. Our aim is to encourage scientists to publish their experimental and theoretical results in as much detail as possible. There is no restriction on the length of the papers. Full experimental and/or methodical details must be provided. Electronic files or software regarding the full details of the calculation and experimental procedure - if unable to be published in a normal way - can be deposited as supplementary material.

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Session 1:
The Biology of ncRNAs – Sponsored by Arraystar Inc.

Session Chairs: Amy Pasquinelli and Reuven Agami

Biology (ISSN 2079-7737) is an international, peer-reviewed, quick-refereeing open access journal of Biological Science published by MDPI online. It publishes reviews, research papers and communications in all areas of biology and at the interface of related disciplines. Our aim is to encourage scientists to publish their experimental and theoretical results in as much detail as possible. There is no restriction on the length of the papers. The full experimental details must be provided so that the results can be reproduced. Electronic files regarding the full details of the experimental procedure, if unable to be published in a normal way, can be deposited as supplementary material.

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Functional Genetic Screens of Regulatory DNA Elements

Reuven Agami

The Netherlands Cancer Institute, Amsterdam.

Regulation of gene expression involves a variety of mechanisms driven by a complex regulatory network of factors. Control of transcription is an important step in gene expression regulation, which integrates the function of cis-acting and trans-acting elements. Among cis-regulatory elements, enhancers and their associated RNAs (eRNAs) recently emerged as widespread and potent regulators of transcription and cell fate decision. Due to lack of genetic tools to investigate enhancers, specific roles of eRNAs and their mechanism of action remained elusive. We therefore developed a CRISPR-based functional genetic-screening tool to uncover functions of regulatory DNA elements. As proof-of-concept experiments we addressed the roles of the enhancer factors p53 and estrogen receptor (ERa) as tumor suppressor and oncogene. I will discuss pros, cons, as well as recent developments of this technology.

1. Lopes, R., G. Korkmaz, and R. Agami, Applying CRISPR-Cas9 tools to identify and characterize transcriptional enhancers. Nat Rev Mol Cell Biol, 2016. 17(9): p. 597-604.2. Korkmaz, G., R. Lopes, A.P. Ugalde, E. Nevedomskaya, R. Han, K. Myacheva, W. Zwart, R. Elkon, and R. Agami, Functional genetic screens for enhancer elements in the human genome using CRISPR-Cas9. Nat Biotechnol, 2016. 34(2): p. 192-8.3. Melo, C.A., J. Drost, P.J. Wijchers, H. van de Werken, E. de Wit, J.A. Oude Vrielink, R. Elkon, S.A. Melo, N. Leveille, R. Kalluri, W. de Laat, and R. Agami, eRNAs are required for p53-dependent enhancer activity and gene transcription. Mol Cell, 2013. 49(3): p. 524-35.

Elucidating the Dark Side of the MicroRNA

Amy E. Pasquinelli

Division of Biological Sciences University of California, San Diego, USA.

The discovery that regulatory RNAs control almost every biological pathway has revolutionized our understanding of gene expression over the past decade. At the forefront, microRNAs (miRNAs) have proven to be an abundant and essential class of RNA molecules in plants and animals. The importance of miRNAs in human biology is highlighted by the increasing recognition that misregulation of specific miRNA pathways contributes to complex diseases, including cancer, heart ailments and neuronal pathologies. Research in the Pasquinelli lab is focused on understanding the molecular mechanisms underlying the biogenesis, specificity and regulatory functions of miRNAs in an endogenous context. New insights into the complicated problem of how miRNAs use limited base pairing to recognize their target sites emerged from combined biochemical and computational approaches aimed at identifying in vivo miRNA-target interactions in the model animal Caenorhabditis elegans. Our findings indicate that, in general, pairing of the miRNA 5'-end is important for functional target interactions and, unexpectedly, sequences in the 3' end of the miRNA convey enhanced specificity.

ZDHHC11 and *ZDHHC11B* Are Novel Critical Components of the Oncogenic miR-150-MYB Network in Burkitt Lymphoma

Joost Kluiver, Anke van den Berg, Agnieszka Dzikiewicz-Krawczyk

Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, The Netherlands.

MYC regulates the expression of protein-coding genes, microRNAs and long non-coding RNAs. Here, we show that overexpression of MYC-repressed miR-150 severely impaired growth of Burkitt lymphoma (BL) cells. AGO2-RIP-Chip revealed novel miR-150 targets *ZDHHC11* and *ZDHHC11B* as the most IP-enriched genes, followed by known miR-150 target *MYB*. *ZDHHC11* and *ZDHHC11B* encode both protein-coding and non-coding transcripts and contain a strikingly high number of miR-150 binding sites, i.e., 18 and 62, respectively. Effective targeting of *ZDHHC11* and *ZDHHC11B* by miR-150 was confirmed by luciferase assay and for the ZDHHC11 protein by Western blot. Furthermore, we identified a circular *ZDHHC11* transcript which contains the miR-150 binding site region and strongly interacts with miR-150. Knockdown of both MYB and *ZDHHC11/ZDHHC11B* significantly impaired growth of BL cells. Moreover, downregulation of *ZDHHC11/ZDHHC11B* also resulted in reduced MYB levels. Finally, genomic deletion of the miR-150 binding site regions also impaired the growth of BL cells. Together, our results demonstrate that elevated MYB levels required for the high proliferative state of BL cells are ensured in two ways. First, MYC represses miR-150 to prevent inhibition of MYB expression. Second, the novel miR-150 targets *ZDHHC11* and *ZDHHC11B* are required for maintaining high MYB levels in BL cells.

Ultra-Conserved Non-Coding Transcript T-UC291 Controls Somatic Tissue Differentiation by Interfering with ACTL6A

Emanule Panatta[1], AnnaMaria Lena[1], Mara Mancini[1], GianGaetano Tartaglia[2], Artem Smirnov[1], George A Calin[3], Gerry Melino[1], Eleonora Candi[1]

[1] University of Rome "Tor Vergata", Dept. Experimental Medicine and Surgery, Rome, Italy. [2] ICREA, Centre for Genetic Regulation, Barcelona, Spain. [3] University of Texas, MD Anderson Cancer Center, Houston, USA.

The mechanisms regulating the switch between epidermal progenitor state and epidermal differentiation are not fully understood. Recent findings indicate that the chromatin remodeler BAF (SWI/SNF) complex and the transcription factor p63 mutually recruit each other to open chromatin during epidermal differentiation. Furthermore, p63 directly regulates the expression of the ATP-dependent chromatin remodeler Brg1 (SMARCA4, catalytic subunit of the BAF complex), and directly interacts with the BAF complex subunit ACTL6a in head and neck squamous cell carcinoma. Here, we identified long non-coding transcripts with an ultra-conserved element, T-UC291, that by physically interacting with ACTL6a modulates chromatin remodeling to allow differentiation. T-UC291 is highly expressed in differentiating keratinocytes and accumulates in the upper layers of human epidermis. Loss of T-UC291 expression inhibits differentiation and promotes the progenitor/undifferentiated state, as evaluated by BrdU incorporation, clonogenic assay and down-regulation of epidermal differentiation complex (EDC) genes. ChIP experiments revealed that upon T-UC291 depletion, ACTL6a is bound to the differentiation gene promoters and inhibits the targeting of BAF complex to differentiation genes, while in the presence of T-UC291, ACTL6a is sequestered, allowing chromatin changes to promote the expression of differentiation genes. Thus, T-UC291 interacts with ACTL6a to modulate chromatin remodeling activity, allowing the transcription of differentiation genes.

A Multiplatform Approach Identifies miR-152 as a Novel Epigenetically Downregulated microRNA in Prostate Cancer

João Ramalho-Carvalho[1], Céline S. Gonçalves[2], Inês Graça[1], David Bidarra[1], Eva Pereira-Silva[1], Maria Inês Godinho[3], Antonio Gomez[4], Manel Esteller[4, 5, 6], Bruno M. M. Costa[2], Rui Henrique[1, 7, 8], Carmen Jeronimo[1, 7]

[1] Cancer Biology & Epigenetics Group – Research Center (CI-IPOP), Portuguese Oncology Institute of Porto (IPO Porto), Portugal. [2] Life and Health Sciences Research Institute (ICVS), School of Medicine & ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Campus de Gualtar, University of Minho, Braga, Portugal. [3] Flow Cytometry Laboratory- Department of Laboratory Medicine, Portuguese Oncology Institute of Porto (IPO Porto), Portugal. [4] Cancer Epigenetics and Biology Program; Bellvitge Biomedical Research Institute; Barcelona, Catalonia, Spain. [5] Institucio Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Catalonia, Spain [6] Physiological Sciences Department, School of Medicine and Health Sciences, University of Barcelona (UB), Catalonia, Spain. [7] Department of Pathology and Molecular Immunology, Institute of Biomedical Sciences Abel Salazar– University of Porto (ICBAS-UP), Portugal. [8] Department of Pathology, Portuguese Oncology Institute of Porto, Portugal.

Prostate cancer (PCa) is a major cause of morbidity and mortality in men worldwide. MicroRNAs are globally downregulated in PCa, especially in poorly differentiated tumors. Nonetheless, the underlying mechanisms are still elusive.

Herein, using combined analysis of microRNAs expression and genomewide DNA methylation, we aimed to identify epigenetically downregulated microRNAs in PCa. We found that miR-152 was underexpressed in PCa and that lower expression levels were associated with promoter hypermethylation. These results were validated in our patient cohort and in TCGA dataset. Functional *in vitro* assays suggest that miR-152 suppresses cell viability and invasion potential, whereas it promotes cell cycle arrest at S and G2/M phases. Finally, *TMEM97*, which is overexpressed in PCa, was identified as a novel miR-152 target gene.

Our findings demonstrate the advantages of using a combinatory approach to identify microRNAs downregulated due to aberrant promoter methylation. MiR-152 downregulation and promoter methylation was found to be prevalent in primary PCa, which impairs its role in control of cell viability, cell cycle regulation and invasion.

Lost in Translation: Long Non-coding RNAs in Metastasis

Qihong Huang

Tumor Microenvironment and Metastasis Program, The Wistar Institute, Philadelphia, USA.

Long noncoding RNAs (lncRNAs) are a novel class of regulatory genes that play critical roles in various processes ranging from normal development to human diseases such as cancer progression. Recent studies have shown that lncRNAs regulate gene expression by chromatin remodeling, transcription, splicing and RNA decay control, enhancer function, and epigenetic regulation. However, little is known about translation regulation by lncRNAs. We identified a lncRNA (treRNA) through genome-wide computational analysis and screening for invasive phenotype in a cell-based assay. We found that treRNA is up-regulated in paired clinical breast cancer primary and lymph node metastasis samples and that its expression stimulates tumor invasion in vitro and metastasis in vivo. Interestingly we found that treRNA down-regulates the expression of the epithelial marker E-cadherin by suppressing the translation of its mRNA. We identified a novel ribonucleoprotein (RNP) complex, consisting of RNA binding proteins (hnRNP K, FXR1, FXR2), PUF60 and SF3B3, that is required for this translational regulatory lncRNA (treRNA) functions. Translational suppression by treRNA is dependent on the 3'UTR of the E-cadherin mRNA. Taken together, our study indicates a novel mechanism of gene regulation by lncRNAs in cancer progression.

MALAT1 and Beyond - Long Non-coding RNAs in Lung Cancer

Gabrijela Dumbovic[1], Jordi Banus[1], Josep Biayna[2], Andreu Alibes[1], Anna Roth[3], Sven Diederichs[3], Sonia Forcales[1], Manuel Perucho[1]

[1]PMPPC. [2]Institute for Research in Biomedicine, Bellinzona, Switzerland. [3]Deutsches Krebsforschungszentrum, Heidelberg, Germany.

Colorectal cancer is a heterogeneous disease characterized by a complex interplay between genetic and epigenetic alterations. Global DNA hypomethylation has been associated to genomic instability, a hallmark of cancer, however the mechanisms linking DNA hypomethylation and chromosomal aberrations are not completely clear. We have identified a macrosatellite repeat frequently demethylated in colorectal tumors; this demethylation is accompanied by changes in histone marks and transcriptional upregulation, leading to accumulation of non-coding transcripts. We are studying whether these ncRNAs could play a role in the oncogenic process, and we also aim to identify contributors to maintain these macrosatellite repeats silenced in normal conditions. Epigenetic modifications in other macrosatellites have been associated to different diseases, however, the molecular underpinnings remain poorly understood. Our studies could shed new light on how a particular macrosatellite may contribute to colorectal and other cancers, and increase our knowledge on the epigenetic control of an enigmatic part of the human genome.

Hyper-Phosphorylation of Argonaute Proteins Affects mRNA Binding and Is Essential for microRNA-Guided Gene Silencing

Daniela Manuela Zeitler, Johannes Danner, Judith Hauptmann, Astrid Bruckmann, Gunter Meister

Biochemistry Center Regensburg (BZR), Laboratory for RNA Biology, University of Regensburg, Regensburg, Germany.

Argonaute proteins associate with microRNAs and are key components of gene silencing pathways. With such a pivotal role in gene silencing, these proteins represent ideal targets for regulatory post-translational modifications. Using quantitative mass spectrometry, we find that a C-terminal Serine/Threonine cluster is phosphorylated at five different residues in human. This conserved hyper-phosphorylation does not affect microRNA binding, localization or cleavage activity of human Ago2. However, mRNA binding is strongly affected. Strikingly, on Ago2 mutants that cannot bind microRNAs or mRNAs, the cluster remains unphosphorylated, indicating a role at late stages of gene silencing. Interestingly, this mutant retains its capacity to produce and bind microRNAs and represses expression when artificially tethered to an mRNA. Altogether, our data suggest that the phosphorylation state of the Serine/Threonine cluster is important for Argonaute–mRNA interactions.

lncRNA RP11-334E6.12 Expression Is Highly Correlated with Increased THY-1 Expression in Chemoresistant Primary Mesothelioma Cells

Jelena Kresoja-Rakic[1], Manuel Ronner[1], Walter Weder[2], Rolf Stahel[3], Emanuela Felley-Bosco[1]

[1] *Laboratory of Molecular Oncology.* [2] *Division of Thoracic Surgery, Zurich University Hospital.*

[3] *Zurich Cancer Center.*

Malignant pleural mesothelioma is an aggressive cancer, which is treated with cisplatin and antifolates. We generated a cisplatin/pemetrexed chemoresistant model of primary mesothelioma cells where we observed increased levels of senescence and autophagy markers. To gain more insight into events occurring during the development of chemoresistance, we analyzed the expression of selected genes at three different time points. Consistent with increased level of senescencence-associated-secretory-phenotype-associated GATA4, we observed a consistent upregulation of *IL-6*. Another gene that was consistently regulated was *THY-1*. Immunofluorescence and flow cytometry analysis indicated an increased fraction of THY-1 positive cells during cis/pem adaptation. Additionally, we detected THY-1 protein by WB in cis/pem adapted cells. Since we realized that we had used primers based on UCSC Genome Browser on Human Feb. 2009 Assembly, which detect both *THY-1* and lncRNA *RP11-334E6.12*, we redesigned primers recognizing individually *THY-1* and lncRNA *RP11-334E6.12* and observed a very strong correlation (r2=0.99) in their expression. Moreover, in the chemoresistant line, changes in lncRNA *RP11-334E6.12* expression doubled the changes in *THY*-1 expression compared to the control line (two-fold increase in slope coefficient). Taken together, this data indicate an enrichment of THY-1 positive cells during chemoresistance development and a possible role for lnc *RP11-334E6.12* in such an increase.

biomedicines

Biomedicines (ISSN 2227-9059) is an international, open access journal aimed at bridging the gap between biological research and clinical practice. Biomedicines provides a forum for all aspects related to biomedicines—medicines that are made from, or contain, biological active substances. Biomedicines publishes cutting-edge research for a diverse audience: academic and clinical researchers, biotechnology companies, healthcare professionals, regulatory and ethical experts, and government and regulatory authorities. Such a multidisciplinary platform should help to promote the translation of basic biomedical research into the clinic to lead to the treatment of diseases.
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Session 2:
Translational Applications of ncRNA as Biomarkers

Session Chairs: Carlo Croce and Menashe Bar-Eli

Cells (ISSN 2073-4409) is an international, peer-reviewed open access journal which provides an advanced forum for studies related to cell biology, molecular biology and biophysics. It publishes reviews, research articles, communications and technical notes. Our aim is to encourage scientists to publish their experimental and theoretical results in as much detail as possible. There is no restriction on the length of the papers. Full experimental and/or methodical details must be provided. There are, in addition, unique features of this journal:

- manuscripts regarding research proposals and research ideas will be particularly welcomed
- electronic files or software regarding the full details of the calculation and experimental procedure - if unable to be published in a normal way - can be deposited as supplementary material
- we also accept manuscripts communicating to a broader audience with regard to research projects financed with public funds

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MicroRNA Dysregulation to Identify Therapeutic Target Combinations for Chronic Lymphocytic Leukemia

Carlo Croce

Department of Cancer Biology and Genetics, The Ohio State University, Columbus, USA.

Chronic lymphocytic leukemia (CLL) is the most frequent adult leukemia in Western countries, occurring as aggressive or indolent disease. More than 80% of patients show genetic aberrations. The most frequent cytogenetic alterations are deletion/inactivation of 13q14 (>55%), deletion of 11q22-23 (18%), trisomy of chromosome 12 (12%), and deletion 17p (7-10%). MicroRNAs are dysregulated in all cancers contributing to cancer pathogenesis. The first demonstration of the role of microRNAs in malignancies was the discovery of the loss of miR-15a and miR-16-1, residing in the deleted region of chromosome 13 in CLL. Indeed, miR-15/16 are downregulated in most patients, primarily due to a deletion on chromosome 13 that occurs in indolent and aggressive cases. Investigation of the targets of miR-15/16 revealed that BCL2, a gene discovered in 1984 and responsible for follicular lymphoma, is a target of miR-15/16. Thus, downregulation of miR-15/16 results in overexpression of BCL2 and malignant transformation. Overexpression of BCL2 in CLLs was detected in most patients. Thus, Steven Fesik and his collaborators at Abbott, developed a protein/protein interaction inhibitor of BCL2. This inhibitor, however, also targeted other members of the BCL2 family, including BclXL, which is essential for platelet survival. More recently, Abbott modified the inhibitor to target only BCL2. This compound, ABT-199 (Venetoclax), was recently approved by the FDA for treatment of patients with relapsed disease with CLL cells harboring deletions in 17p. Venetoclax is able to induce lysis of CLL cells and complete responses without detectable minimal residual disease. Dr. Kipps' laboratory and others showed that the receptor tyrosine kinase-like orphan receptor 1 (ROR1) is an onco-embryonic antigen found on most CLL B-cells, but not on normal B-cells or normal adult tissues, except a small subgroup of B-cell precursors named hematogones. Additionally, antibodies targeting ROR1 can inhibit ROR1 induced cell growth in cells expressing high-level of ROR1 and TCL1 which is associated with aggressive CLL. Thus, Dr. Kipps laboratory developed an anti-ROR1 antibody (Cirmtuzumab), for clinical trials. Evaluation of ROR1 on CLL cells from 1568 cases showed that levels of ROR1 varied between patients. Whereas the majority of cases expressed detectable ROR1, 5-10% of cases expressed negligible levels of ROR1, comparable to that of normal B-cells. Moreover, CLL cases with higher expression of ROR1 were associated with more aggressive disease and shorter overall survival. Based on these observations, ROR1 appears to be a good target for novel therapies for CLL patients. Thus, we examined whether microRNA signatures could be identified in samples from CLL patients with B cells expressing high or low levels of ROR1 as previously defined.

RNA Editing and Melanoma Metastasis

Einav Shoshan[1], Aaron K. Mobley[1], Russell R. Braeuer[1], Takafumi Kamiya[1], Li Huang[1], Mayra Vasquez[1], Ahmad Salameh[2], Ho Jeong Lee[1], Sun Jin Kim[1], Cristina Ivan[3], George A. Calin[4], Anil K. Sood[1, 3], Patrick Hwu[5], Jeffrey E. Gershenwald[6], Gal Markel[7, 8], Isaiah J. Fidler[1], Menashe Bar-Eli[1]

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Although recent studies have shown that adenosine-to-inosine (A-to-I) RNA editing occurs in microRNAs, its effects on tumor growth and metastasis are not well understood. We present evidence of CREB-mediated low expression of ADAR1 in metastatic melanoma cell lines and tumor specimens. Re-expression of ADAR1 resulted in the suppression of melanoma growth and metastasis in vivo. Consequently, we identified 3 miRs undergoing A-to-I editing in the low-metastatic melanoma cell lines but not in highly metastatic. One of these miRs, miR-455 has two A-to-I RNA editing sites. The biological function of edited miR-455 is different from the unedited form. Indeed, w.t. miR-455 promotes melanoma metastasis via inhibition of the tumor suppressor gene CPEB1. Moreover, w.t. miR-455 enhances melanoma growth and metastasis in vivo while the edited form inhibits these features. TCGA analysis confirmed accumulation of wild-type miR-455 in metastatic melanoma lesions. On the other hand, expression of the PARVA oncogene thus prevents melanoma progression. These results demonstrate a previously unrecognized role of RNA editing in melanoma Progression.

Shoshan et al…Menashe Bar-Eli, Nat Cell Biol. 2015 Mar;17(3):311-21. doi: 10.1038/ncb3110. Epub 2015 Feb 16. PMID:25686251

miRNAs Modulating Melanoma Cell Invasion

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Melanoma is the most lethal form of skin cancer characterized by frequent metastasis, occurring already at early stage of disease. So far, standard therapies have yielded only moderate success, calling for novel therapeutic strategies. Therapeutic targeting of mRNAs encoding proteins functionally involved in the regulation of melanoma cell invasion might represent a promising treatment approach. MiRNAs are small noncoding RNAs with regulatory function in cellular gene expression. In cancer cells, miRNAs are often aberrantly expressed, resulting in loss of their regulatory function. Recently, we unraveled several miRNAs affecting melanoma cell invasion and identified miR339-3p as a new tumor-suppressor miRNA (Weber et al., Cancer Res 2016). Here, we show that miR-193b, miR-30c-1*, and miR-339-3p inhibit, whereas miR-576-5p accelerates invasion of various melanoma cell lines. Using gene expression profiling, we identified potential targets of these miRNAs and validated regulation of BCL9 and STMN1 expression by the miRNAs identified. The opposing effects of miR-193b, miR-30c-1* and miR-576-5p on BCL9 expression might account for the different invasion phenotypes observed. Luciferase reporter-assay proved direct interaction of miR-339-3p and miR-193b with the 3' UTR of MCL1. Thus, miR-193b and miR-399-3p could be confirmed as tumor-suppressive miRNAs, whereas miR-576-5p was identified as a novel oncomiR.

Noncoding RNA Function and Regulation in Animal Development

Helge Grosshans

Friedrich Miescher Institute for Biomedical Research (FMI) Basel, Switzerland.

The past 25 years have seen a noncoding RNA revolution that was initiated by the discovery of the *lin-4* microRNA (miRNA) in the nematode *Caenorhabditis elegans*. Here, I will report how we have used *C. elegans* to dissect rules of miRNA target engagement, regulation, and physiological function *in vivo* through genetic, biochemical and genomics approaches. I will focus on our recent identification and ongoing characterization of a novel physical interaction partner of Argonaute, the miRNA-binding protein that is at the core of the miRNA-induced Silencing Complex (miRISC). The new factor, important for animal fertility, binds Argonaute loaded with miRNAs but devoid of the GW182 miRISC effector protein. Hence, we propose, and test in ongoing experiments, that it plays a role in Argonaute target binding, recycling, or quality control.

Role of Exosomal miRNAs in the Biology of the Tumor Microenvironment

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Exosomes are small extracellular vesicles involved in inter-cellular communication. Their cargo consists of proteins, nucleic acids and lipids that can be functionally shuttled from one cell to another. We discovered that microRNAs within exosomes can be secreted by cancer cells and up-taken by surrounding Tumor-Associated Macrophages (TAMs) expressing Toll-like receptor 8 (TLR8). We showed that miR-21 can directly bind to TLR8 and activate this receptor downstream signaling leading to increased secretion of exosomal miR-155 from TAMs to cancer cells, increasing resistance to chemotherapy. This lecture will focus on this new mechanism of inter-cellular interaction and will show how exosomal miRNAs released by cancer cells and by the immune cells can be exploited for diagnostic and therapeutic purposes.

Epigenetic Biomarkers of Prognosis in Stage IIA Colon Cancer

Manuela Ferracin

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Adjuvant therapy is a systemic treatment administered after primary tumor resection with the aim of reducing the risk of relapse and death in cancer patients. Generally, adjuvant treatment is recommended for stage III and 'high-risk' stage II colorectal cancers, although there is no evidence for a predictive marker regarding the benefit of adjuvant chemotherapy. Therefore, the choice to administer adjuvant therapy after surgery in stage II colon cancer is still highly debated. This is specifically true for stage IIA (T3N0) patients, whose estimated recurrence rate is 15-20% in the absence of any further therapy after resection of the primary tumor. With the aim to find a potentially predictive biomarkers, we analyzed the global methylation profile and microRNA expression profile of T3N0 FFPE colon samples. We identified specific epigenetic modifications that were able to predict recurrence in chemotherapy-naïve patients and represent candidate prognostic biomarkers.

Alterations of MicroRNAs throughout the Malignant Evolution of Cutaneous Squamous Cell Carcinoma: The Role of miR-497 in Epithelial-to-Mesenchymal Transition of Keratinocytes

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Skin carcinogenesis is known to be a multi-step process with several stages along its malignant evolution. We hypothesized that transformation of normal epidermis to cutaneous squamous cell carcinoma (cSCC) is causally linked to alterations in miRNA expression. For this end, we decided to evaluate their alterations in the pathologic states ending in cSCC. Total RNA was extracted from FFPE biopsies of five stages along the malignant evolution of keratinocytes towards cSCC: normal epidermis, solar elastosis, actinic keratosis KIN1-2, advanced actinic keratosis, KIN3 and well differentiated cSCC. Next generation small RNA sequencing was performed. We found that 18 miRNAs are over-expressed and 28 miRNAs are under-expressed in cSCC compared to normal epidermis. miR-424, miR-320, miR-222 and miR-15a showed the highest fold change among the over-expressed miRNAs. miR-100, miR-101 and miR-497 showed the highest fold change among the under-expressed miRNAs. The heat map of hierarchical clustering analysis of significantly changed miRNAs and principle component analysis disclosed that the most prominent change in miRNAs expression occurred in the switch from “early” stages—normal epidermis, solar elastosis and early actinic keratosis—to the “late” stages of epidermal carcinogenesis—late actinic keratosis and cSCC. We found several miRNAs with "stage specific" alterations while others display clear “gradual” alterations, either progressive increase or decrease in expression along the malignant evolution of keratinocytes. The observed alterations in miRNAs involved the regulation of AKT/mTOR or involved epithelial-to-mesenchymal transition. We chose to concentrate on the evaluation of the molecular role of miR-497. We found that it induces reversal of epithelial-to-mesenchymal transition. We proved that SERPIN-1 is its biochemical target.

The present study allows us to further study the pathways which are regulated by miRNAs along the malignant evolution of keratinocytes towards cSCC.

Session 3:
Bioinformatics of ncRNAs

Session Chairs: Isidore Rigoutsos and John S. Mattick

Editor-in-Chief: Prof. Dr. Maurizio Battino - Department of Odontostomatologic and Specialized Clinical Sciences, Sez-Biochimica, Faculty of Medicine, Università Politecnica delle Marche, Via Ranieri 65, 60100 Ancona, Italy

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Transcriptomic Heterogeneity: Known and Novel Short Non-Coding Regulatory RNAs That Depend on Sex, Population Origin, Tissue, and Disease

Isidore Rigoutsos

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Non-coding RNAs (ncRNAs) are molecules that do not code for proteins but regulate cell physiology via multifaceted mechanisms. MicroRNA (miRNA) isoforms, known as isomiRs, and tRNA-derived fragments, known as tRFs, are two novel families of such ncRNAs. In this presentation, I will present recent findings by my group and discuss their ramifications for basic and applied Research.

IsomiRs are variants of the same mature miRNA; any two isomiRs of the same miRNA differ in their endpoints only by a few nucleotides. Fast accumulating evidence shows that what was considered to be a single “miRNA” is actually a cloud of co-existing isomiRs that are produced from the same locus. Analogously, tRFs are short molecules that arise from precursor and full-length mature tRNAs and co-exist simultaneously with the mature tRNA. Both isomiRs and tRFs have been shown to enter the RNAi pathway, and, thus have regulatory roles.

By analyzing many datasets from healthy individuals and cancer patients, we found that isomiRs and tRFs represent constitutive molecules. Moreover, we found that the abundance profiles for both isomiRs and tRFs depend on an individual's sex, population origin, and race as well as on tissue, tissue state, and disease subtype.

IsomiRs and tRFs, and their dependence on variables that had not been considered previously in this context, are an instance of "transcriptomic heterogeneity" (TH). TH manifests itself when a given segment of DNA produces different RNA products, either in different tissues of the same individual, or in the same tissue of individuals who differ in sex, population origin, race, age, disease subtype, etc. In TH, disease is associated with differences in the RNA molecules that are produced from a given segment of DNA. This is unlike genomic heterogeneity where disease is associated with variations in the DNA template.

The 3' UTR Landscape of Human Cancers

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High-throughput sequencing has uncovered a tremendous complexity of mammalian transcription. However, whereas many studies have been dedicated to the mapping of alternative promoters and to their functional exploration, the equally large diversity of polyadenylation sites has been much less studied. Alternative polyadenylation is linked to basic cellular programs, as the length of 3' untranslated regions (3' UTRs) increases during cell differentiation and decreases upon lymphocyte activation or malignant transformation. Strikingly, the upstream regulators and the functional relevance of 3' UTR forms are poorly understood. Starting from our finding that the cleavage factor I, a core component of the human cleavage and polyadenylation complex, is a key regulator of 3' UTR lengths, we have developed computational methods to infer regulators of 3' end processing based on mRNA sequencing data. In particular, we have studied the data from The Cancer Genome Atlas to uncover poyadenylation site changes in human cancers.

Fatal Imperfections: Determinants of miRNA Target Specificity

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MicroRNAs (miRNAs) are small RNAs of about 22 nucleotides that silence target messenger RNAs by binding to partially complementary regions in their 3' UTRs. Base pairing of the so-called seed sequence of a miRNA is particularly important and often thought to be sufficient for gene silencing. miRNAs with the same seed sequence are therefore grouped into "families" and supposed to share the same targets. However, it is unclear whether the seed sequence is the only determinant for target repression. To understand what establishes miRNA target specificity in a physiological setting, we used quantitative microscopy to assess the repression of miRNA target reporter genes in the nematode *C. elegans*. We observed specificity among the members of the *let-7* family (*let-7 proper*, miR-48, miR-84 and miR-241) *in vivo*, and found that it depended on target site architecture. Both sequences bound by the seed and those bound by other parts of the miRNA contributed to family member specificity. Strikingly, for certain target site architectures, changes in the miRNA levels can modulate the specificity. By contrast, other architectures can 'lock' specificity such that it cannot be overcome by miRNA over-expression. We are currently testing the physiological relevance of miRNA family member-specific regulation by using genome editing to redirect an endogenous target from exclusive repression by *let-7 proper*.

miRNAtools: Advanced Training Using the miRNA Web of Knowledge

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miRNAs are small non-coding RNAs, that act as negative regulators of the genomic output. Their intrinsic importance within cell biology and human disease is well known. Their mechanism of action based on the base pairing binding to their cognate targets, has helped the development of many computer applications for the prediction of miRNA target recognition. More recently, many other computer applications have appeared with the objective of studying miRNA roles in many contexts, trying to dissect and predict their functions in a specific biological process. Learning about miRNA function requires practical training in the use of specific computer and web-based applications that are complementary to the wet-lab studies.

In the last six years, we have been involved in the organization of advanced training initiatives about the in silico functional analysis of miRNAs and non-coding RNAs, for students ranging from the post-graduate to the post-doctoral level. In order to guide the learning process about miRNAs, we have created miRNAtools (http://mirnatools.eu), a web repository of miRNA tools and tutorials. This page is a compilation of tools to analyze miRNAs and their regulatory actions; it aims to collect and organize the information that is disperse in the web. It harbors sections compiling web-sites and tools for miRNA analysis, including general purpose databases, specialized databases, single and multiple target prediction algorithms, tools for pathway and integrative analysis, and software devoted to the analysis of miRNA expression by next-generation sequencing. All the sections are introduced by practical and personal assessments regarding the advantages and problems of each tool, and its applicability. The miRNAtools webpage is completed by a collection of tutorials that can be used by students and tutors engaged in advanced training courses. The tutorials follow the rationale of the analysis of the function of a particular miRNA, starting from its nomenclature and genomic localization and finishing by assessing its involvement in specific cellular pathways and functions.

Cancer Driver Long Noncoding RNA Discovery in the Pan-Cancer Analysis of Whole Genomes (PCAWG) Collaboration

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Do mutations in long noncoding RNAs contribute to the evolution of tumours? We can address this, for the first time, using whole tumour genome sequences. The PCAWG collaboration of the International Cancer Genome Consortium (ICGC) has sequenced thousands of entire tumour genomes and mapped their mutations. As part of this effort, we have developed an approach called "ExInAtor" to identify driver lncRNAs based on their mutational load. I will present the latest results from this analysis, including known and novel lncRNAs. In order to benchmark results, we have also created a reference collection of true positive genes, the "Cancer LncRNA Census" (CLC). In addition to its utility in method development, CLC also reveals that cancer lncRNAs have a series of unique genomic features and evidence for deeply-evolutionarily conserved functions. In summary, PCAWG genomes are revealing the landscape of mutated driver lncRNAs in tumours.

Exons Are the Modular Unit of Structure-Function in Regulatory RNAs

John S. Mattick

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The mammalian genome is pervasively transcribed to produce not only a suite of mRNAs specifying a relatively stable proteome but also a plethora of short and long noncoding RNAs (lncRNAs) with regulatory functions. Many if not most long lncRNAs are expressed in highly restricted patterns, commensurate with their emerging role as epigenetic guides, which has to date resulted in low sequence coverage and difficulties in building accurate transcript models. We have used RNA CaptureSeq to obtain high-depth, high-resolution coverage of transcription from human ch21 and its syntenic regions in mouse, which has revealed, surprisingly, that noncoding exons (in both mRNAs and lncRNAs) are universally alternatively spliced. In parallel, we have found that evolutionarily conserved RNA structures, of which there are thousands of families, are overwhelmingly confined within exons, with few crossing exon–exon boundaries. Both observations imply, and together force the conclusion, that the unit of structure-function in regulatory RNAs is the exon, combinations of which can create an enormous diversity of isoforms presumably required to organise a precise 4-dimensional developmental ontogeny. This modular structure also provides enormous flexibility for adaptive radiation, and a firm basis for parsing the evolutionary history and the functional architecture of regulatory RNAs. It further suggests, given our previous observations, that exons are preferentially located in nucleosomes and that alternatively spliced exons are pre-organised in transcription-splicing complexes, that exons are the atomic unit of epigenetic regulation.

Chromatin-Release Is Important for Long NcRNA Function

Ulf Andersson Ørom

Long non-coding RNA Research Group Max Planck Institute for Molecular Genetics, Germany.

Long non-coding RNAs (ncRNAs) are emerging as important regulators of numerous biological processes, involving examples of both positive and negative regulation of transcription. Activating long ncRNAs are transcribed from enhancer-like regions and regulate adjacent target gene expression apparently in *cis*. Long ncRNAs are often enriched in the nucleus and at chromatin but whether chromatin-release plays a functional role is unknown. We show that long ncRNAs engaged in strong chromatin interactions are less enriched in the chromatin fraction, suggesting a functional involvement of chromatin-release of long ncRNAs in transcriptional regulation. To study this further, we identify the long ncRNA *A-ROD*, an activating regulator of the *Wnt* signaling inhibitor *DKK1*. We show that *A-ROD* enhances transcription elongation of *DKK1* in an RNA-dependent manner and this regulation involves the recruitment external proteins to the *DKK1* promoter. We propose, that the activating function depends on the release of A-ROD from chromatin, in agreement with a quasi-*cis* mechanism of action. Our data suggest that the release of a subset of long ncRNAs is important for their function, adding a new mechanistic perspective to the subcellular localization of long ncRNAs, and identify a functional regulatory interaction mediated by *A-ROD* in the transcription activation of *DKK1*.

Epigenetics and Epitranscriptomics of Non-Coding RNAs in Human Cancer

Manel Esteller

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The relevance of the non-coding genome to human disease has mainly been studied in the context of the widespread disruption of microRNA (miRNA) expression and function that is seen in human cancer. However, we are only beginning to understand the nature and extent of the involvement of non-coding RNAs (ncRNAs) in disease. Other ncRNAs, such as PIWI-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs), transcribed ultraconserved regions (T-UCRs) and large intergenic non-coding RNAs (lincRNAs) are emerging as key elements of cellular homeostasis. Along with microRNAs, dysregulation of these ncRNAs is being found to have relevance for tumorigenesis and other human diseases, such as Rett syndrome. Furthermore, different types of ncRNAs are able to regulate each other. Furthermore, chemical modification of ncRNAs can change their functions and targets. The described many activities of these molecules have awakened a great interest in therapeutic strategies to counteract the perturbations of ncRNAs.

Session 4:
ncRNA Therapeutics

Session Chairs: Frank J. Slack and Massimo Negrini

Editor-in-Chief: Prof. Dr. J. Peter W. Young - Department of Biology, University of York, Heslington, York YO10 5DD, UK

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MicroRNA-Based Therapeutics in Cancer

Frank J. Slack

Professor, Pathology, Harvard Medical School Professor, Pathology, Beth Israel Deaconess Medical Center, Boston, USA.

MicroRNAs are small non-coding RNAs that regulate gene expression to control important aspects of development and metabolism such as cell differentiation, apoptosis and lifespan. miR-21, miR-155, *let-7* and miR-34 are microRNAs implicated in human cancer. Specifically, human *let-7* and miR-34 are poorly expressed or deleted in lung cancer, and over-expression of *let-7* or miR-34 in lung cancer cells inhibits their growth, demonstrating a role for these miRNAs as tumor suppressors in lung tissue. *let-7* and miR-34 regulate the expression of important oncogenes implicated in lung cancer, suggesting a mechanism for their involvement in cancer. We are focused on the role of these genes in regulating proto-oncogene expression during development and cancer, and on using miRNAs to suppress tumorigenesis. In contrast, miR-21 and miR-155 are oncomiRs and up-regulated in many cancer types. We are also developing effective strategies to target these miRNAs as a novel anti-cancer approach. Lastly we are examining the non-coding portions of the genome for mutations and variants that are likely to impact the cancer phenotype. We have successfully resequenced the 3'UTRome and microRNAome from cancer patients with a family history of cancer.

About Chomsky, Non-Coding RNA Structure and Cancer Patient's Treatment

George A. Calin

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More than three decades after the identification of the first non-codingRNA (ncRNA) in the human genome, the H19 gene, the number of annotated ncRNAs exceeds that of protein coding genes by one order of magnitude and the gap is continuously expanding. An even larger set of non-coding transcripts, many primate-specific, is still awaiting annotation. These discoveries have created a compelling need to understand the structure-function relationships that underline the biological roles of ncRNAs. We propose to review the timely topic of how the structure of ncRNA informs its function by focusing on the domain structure of ncRNAs. The premise of this presentation is the growing realization that ncRNAs, like large proteins, have a multidomain architecture, raising the questions of: 1) how function localizes to specific domains within ncRNAs; 2) what roles these domains play in normal and aberrant cellular physiology; 3) whether their function can be redirected for therapeutic advances. The proposed review will present recent advances in mapping the domain structure and function of non-coding RNAs; how motifs and domains can be identified within ncRNAs; and how their identification can be used for understanding new facets of signaling pathways and in the development of new therapeutic approaches to treating human disease. We will suggest that the spectrum of ncRNAs motifs (words) is organized in sentences (the complete ncRNA structure) with a functional (signaling output) meaning, and propose an analogy with Chomsky's grammar to illuminate our understanding of the functional and structural organization of these transcripts.

Role of miR-125a in Hepatic Carcinogenesis

Nicoletta Potenza[1], Nicola Coppola[1], Giorgio de Stefano[2], Silvia Zappavigna[1], Marta Panella[1], Lorenzo Onorato[1], Nunzia Farella[2], Nicola Mosca[1], Carmine Minichini[1], Mario Starace[1], Michele Caraglia[1], Aniello Russo[1]

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Our studies are focused on the role of hsa-miR-125a-5p (miR-125a) in the development of hepatocellular carcinoma (HCC) for two reasons: 1) it downregulates oncogenic proteins in several types of tumors, and 2) it is implicated in the hepatocyte/hepatitis B virus interaction, with hepatitis B being one of the most important risk factors for HCC development. We first showed that miR-125a inhibits proliferation of cultured HCC cells by p21/p27-dependent cell cycle arrest in G1. Then, the analysis of a number of miR-125a validated targets relevant to the antiproliferative activity revealed that sirtuin-7, matrix metalloproteinase-11, Zbtb7a, and c-Raf were downregulated. Interestingly, miR-125a was found to be induced by sorafenib, the antitumor drug for treatment of advanced HCC, and to be part of its mechanism of action. The expression of miR-125a was then evaluated in 55 tumor biopsies of HCC and in matched adjacent non-tumor liver tissues, showing its down-regulation in 80% of tumors with a mean 4.7-fold decrease. Sirtuin-7, matrix metalloproteinase-11 and c-Raf were conversely up-regulated by 2.2-, 3-, and 1.7-fold, respectively. Finally, we studied the molecular mechanisms governing miR-125a expression and identified its transcription promoter. Overall, these data support a tumor suppressive role for miR-125a.

Multiple Approaches for miRNA-based Therapies of Cancer

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The abnormal expression of microRNAs represents a key mechanism that drives tumorigenesis and may also potentially identify targets for anti-tumor therapy. In the course of the years, we have investigated this area of study. In human HCC, among consistently deregulated miRNAs, microRNA-221 is up-regulated in about 80% of cases and miR-199 is down-regulated in virtually all. These findings were at the basis for investigating miRNA-based therapeutic approaches. We developed a Conditionally Replicating Adenovirus dependent on the expression of miR-199 (CRAd-199). This virus was able to replicate in cells lacking the expression of miR-199, like HCC cells, but its replication was impaired in cells expressing high level of miR-199, like normal liver parenchima. This virus demonstrated a very good in vivo anti-tumor activity. We also developed a transgenic mouse that expresses high level of miR-221 in the liver. The mouse becomes strongly predisposed to liver cancer. Spontaneous tumors occur in about 50% of male mice; in addition, 100% of DENA or CCl4 treated mice develop liver cancer significantly earlier than wild type mice. This model was used to test of anti-miR-221 and miR-199, which demonstrated a good anti-tumor efficacy. In addition, in the course of these studies, we tested methods for in vivo delivery of small oligonucleotides. Based on this experience, we approached also the testing of miRNA molecules as therapeutics in a mouse model of chronic lymphocytic leukemia. In this model, we investigated the anti-leukemic activity of several miRNAs. miR-26a emerged as the most effective in contrasting the growth of leukemic cells in vivo. Delivery of miRNA molecules to spleen, where leukemic cells accumulate, was improved by the use of lipid nanoparticles, whose efficiency was further increased by the conjugation with an anti-CD38 antibody, able to confer specificity for the leukemic cells. The studies proved that basic information on miRNA expression can be translated into possibly useful therapeutic approaches and miRNA-based molecules have the potential for being used as therapeutics. To become clinically valuable for cancer therapy, stronger anti-tumor effects need to be consistently achieved.

Transcriptome-Wide Mapping of the miR-122 Targetome Revealed Its Mechanistic Role in the Maintenance of Liver Homeostasis and Suppressing Hepatocarcnogenesis

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Liver cancer has the second highest mortality rate among all malignancies, which implies a lack of molecular understanding of disease development and progression necessary to combat this deadly disease. miR-122 is a conserved liver-specific miRNA, which maintains metabolic homeostasis, suppresses tumor development, and promotes HCV replication. Down regulation of miR-122 expression is associated with loss of hepatic phenotype, gain of malignancy-associated characteristics, and poor prognosis in hepatocellular carcinoma (HCC) patients. Recent in vivo studies in miR-122 knockout (KO) mice have established it as a bona fide tumor suppressor. These data suggest that it is critical to identify the miR-122 targetome in the liver to better understand its molecular functions. To biochemically identify miR-122 targets in the liver transcriptome, we used an unbiased high-throughput method, known as Ago-dHiTS-CLIP (Ago-CLIP). To this end, in collaboration with Dr. Darnell's laboratory, we performed Ago-CLIP analysis of livers of 6 week-old control (Mir122fl/fl) and miR-122 KO (Mir122-/-) mice as well as benign human livers expressing relatively high levels of miR-122 and HCCs from the same patient exhibiting reduced miR-122 level. Ago-CLIP data demonstrated that the majority of miR-122 sites are in the coding exons followed by 3'-UTRs along with sites in the introns, 5'UTRs, transposable elements etc. Motif analysis revealed enrichment of a large number miR-122 targets with canonical (6, 7 and 8 mer) binding sites. In addition, a novel miR-122-dependent but non-canonical G-bulged motif were also identified both in the mouse and human livers. Surprisingly, PhyloP scores revealed that the majority of miR-122 targets are species-specific. Cumulative fraction analysis of the RNA-seq data revealed significant de-repression of only canonical 3'UTR and CDS targets in miR-122 KO livers, suggesting these are functional miR-122 targets. Consistent with our findings in miR-122 KO mice, we found that both canonical and G-bulged miR-122 3'UTR sites were significantly less bound with miR-122 in tumor samples. Only 965 (~20%) of human targets identified shared overlap between the two species. Analysis of Liver and Hepatocellular Carcinoma (LIHC) data from The Cancer Genome Atlas (TCGA) revealed that the majority of these common targets are upregulated in primary HCCs, and alterations in 26 targets are predictive of overall patient prognosis. BCL9, an exclusive CDS target of miR-122, and a critical component of Wnt/β-catenin signaling, was found to be significantly predictive of patient survival, and may play a key role in liver tumor progression. In vitro studies using miR-122 KO, BCL9 KO and double KO cells showed that miR-122 indeed modulates β-catenin transcriptional activity by targeting its co-activator BCL9. Collectively, these results demonstrate that Ago-CLIP technique identified many novel targets in the mouse and human livers some of which may play a causal role in hepatocarcinogenesis.

Development of RNA Aptamers for Targeting B-cell-derived Malignancies

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B-cell chronic lymphocytic leukemia (B-CLL) and multiple myeloma (MM) are the most common forms of adult leukemia in the Western world. Despite recent advance in the therapeutic management of these malignancies, usually, after an initial good response to the treatments, patients progressively become refractory, showing high rate disease recurrence. Therefore, B-CLL and MM remain incurable and the development of new therapeutic options for these patients is a main challenge in cancer research. Nucleic acid aptamers represent a very attractive class of high affinity ligands with the potential to inhibit disease-associated proteins. They show many advantages as therapeutic agents, including low toxicity, high specificity and adequate stability in biological fluids. Moreover, internalizing aptamers provide effective delivery carriers for the selective diffusion of secondary therapeutic agents, reducing the occurrence of off-target effects.
Here we select internalizing aptamers directed against surface markers of malignant B-cells, by a cell-SELEX (Systematic Evolution of Ligands by Exponential enrichment) approach properly modified in our laboratory. The final aim of our project is to develop these aptamers for the selective delivery of RNA-based therapeutics for B-CLL and MM targeted therapy.
Acknowledgements: this work was supported by funds from: AIRC # 13345 and # 9980

A Specific Long Non-Coding RNA Expression Signature Defines the Philadelphia-like B-Cell Acute Lymphoblastic Leukemia Subtype

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Emerging evidence suggests that long non-coding RNAs (lncRNAs) play a major role in cancer development. This study aims to explore the lncRNA landscape of Philadelphia-like (Ph-like) B-cell Precursor Acute Lymphoblastic Leukemia (BCP-ALL), a newly identified high-risk subtype of ALL. We performed whole transcriptome sequencing (RNA-seq) and DNA-methylation arrays on 82 BCP-ALL samples (Ph-like n=21; non-Ph-like n=61) of patients from the German Multicenter Study Group ALL (GMALL) trials. We identified 442 (fold change <=> +-1.5, FDR<=0.05) Ph-like specific lncRNAs. Hierarchical clustering on these lncRNAs revealed a robust cluster associated with the Ph-like defined protein-coding (PC) expression signature. Analyzing the co-expression of lncRNAs with cis PC genes (p.val<=0.05) showed enrichment of key signaling pathways such as JAK-STAT, PI3K-Akt, Hippo and cytokine–cytokine pathways. We looked into the epigenetic regulation of Ph-like associated lncRNAs by investigating their DNA-methylation pattern. We identified 24 lncRNAs with hyper- and hypo-methylated (p.val<=0.01) regions and showed anti-correlation to their expression levels (p.val<=0.05) that indicates epigenetic regulation. Our study not only provides a defined signature of Ph-like specific lncRNAs and their functions but also underscores their epigenetic regulation. Thus, our work gives insights into potential functions of Ph-like specific lncRNAs in BCP-ALL.

Session 5:
ncRNA Technologies

Session Chairs: Thomas Schmittgen and Manel Esteller

Journal of *Developmental Biology*

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microRNAs Shape Plasticity of Pancreatic Acini

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Despite numerous profiling studies that report differential miRNA expression in pancreatic ductal adenocarcinoma (PDAC), it remains unclear what, if any, role miRNAs play during PDAC initiation and/or progression. This study was undertaken to examine the function of three pancreas enriched miRNAs towards the development of PDAC. Studies in mice over the past decade have shown that PDAC arises following transdifferentiation of the pancreatic acini into ductal like cells, i.e. the process of acinar ductal metaplasia or ADM. In the context of mutant Kras, ADM gives rise to PanINs which are followed by invasive PDAC. The molecular events describing ADM are still under active investigation. We hypothesize that miR-216a, -216b and -217 maintain the acinar phenotype and upon their loss induce ADM, PanINs and PDAC. CRISPR/CAS genome editing was used to generate the three germline miRNA knockout mice (miR-216a, -216b and -217). To assess a phenotype of the knockouts, mice were challenged with the cholecystokinin analog careulein or crossed with mice harboring Kras conditionally activated in the pancreas. Validation of the knockouts was performed by DNA sequencing and qRT-PCR. An in vitro ADM assay was performed by culturing pancreatic acinar cells on collagen/TGFα. Increased in vitro acinar-ductal transdifferentiation was observed when the isolated acinar cells from the miRNA knockouts are cultured on collagen. Increased apoptosis and duct formation was evident when the knockout mice were subjected to caerulein induced acute pancreatitis. miRNA-216a and miR-216b knockout mice were also found to develop pancreatic duct glands following careulein injection. The bigenic offspring of miRNA knockout and EL-Kras^{G12D} cross dramatically accelerates the development of Kras driven acinar-ductal metaplasia and formation of PDA precursor lesions. These results suggest that miRNA-216/217 is required for maintaining the acinar fate and for regeneration following pancreatic injury. We propose that these miRNAs act as tumor suppressors by repressing ductal metaplasia and thereby limit the extent of PDAC progression.

Tools for lncRNA Research in Cancer

Jo Vandesompele

Chair of the Cancer Research Institute, Ghent University, Belgium.

The human genome is pervasively transcribed, giving rise to an increasing number of long non-coding RNA genes. Most of these genes are novel or poorly characterized, and their relevance in human health and disease remains elusive. In our lab, we have developed various tools to study lncRNAs, amongst others to assess their role in cancer. As such, we are looking for novel biomarkers and therapeutic targets. I will describe various tools and ongoing research programs, including a comprehensive annotated catalog of human lncRNAs (LNCipedia), a targeted screen for focal lncRNA copy number alterations, a web tool for antisense oligonucleotide design, Zipper plot to visualize the transcriptional activity of lncRNAs in their genomic context, decodeRNA functional context mapping, and probe based lncRNA capture sequencing in body fluids.

The microRNA-449 Family Inhibits TGF-β-mediated Liver Cancer Cell Migration by Targeting *SOX4*

Beate Vajen[1], Maria Sandbothe[1], Reena Buurman[1], Engin Gürlevik[2], Nicole Reich[1], Luisa Greiwe[1], Vera Schäffer[1], Marlies Eilers[1], Florian Kühnel[2], Alejandro Vaquero[3], Thomas Longerich[4], Peter Schirmacher[5], Michael Manns[2], Illig Thomas[1], Brigitte Schlegelberger[1], Britta Skawran[1]

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Introduction: Modulation of microRNA expression is considered for treatment of hepatocellular carcinoma (HCC). Therefore, we characterized the epigenetically regulated microRNA-449 family (miR-449a, miR-449b, miR-449c) with regards to its functional effects and target genes in HCC.
Methods: After transfection of miR-449a, miR-449b, and/or miR-449c, tumor-relevant functional effects were analyzed using *in vitro* assays and a xenograft mouse model. Binding specificities, target genes, and regulated pathways of each microRNA were identified by microarray analyses. Target genes were validated by luciferase reporter assays and expression analyses *in vitro*. Furthermore, target gene expression was analyzed in 61 primary human HCCs compared to normal liver tissue.
Results: Tumor suppressive effects, binding specificities, target genes, and regulated pathways of miR-449a and miR-449b differed from those of miR-449c. Transfection of miR-449a, miR-449b, and/or miR-449c inhibited cell proliferation and migration, induced apoptosis, and reduced tumor growth to different extents. Importantly, miR-449a, miR-449b, and, to a lesser degree, miR-449c directly targeted *SOX4*, which codes for a transcription factor involved in epithelial-mesenchymal transition and HCC metastasis, and thereby inhibited TGF-β-mediated cell migration.
Conclusions: This study provides detailed insights into the regulatory network of the epigenetically regulated microRNA-449 family and, for the first time, describes distinct tumor suppressive effects and target specificities of miR-449a, miR-449b, and miR-449c. Our results indicate that particularly miR-449a and miR-449b may be considered for miRNA replacement therapy to prevent HCC progression and metastasis.

An Improved Algorithm for Antisense LNA™ GapmeR Design

Jörg Krummheuer, Niels Montano Fransen, Johnathan Lai, Asli Özen, Jesper Culmsee Tholstrup, Dhani Saputra, Niels Tolstrup, Peter Mouritzen

Exiqon / Qiagen Vedbaek, Denmark.

One of the most basic yet powerful approaches to study lncRNAs is loss of function analysis, but since many lncRNAs are nuclear retained or have long residence time in the nucleus, these are difficult to target by classical RNAi-based methods.

We have developed single-stranded LNA™-enhanced antisense oligonucleotides (ASOs, also known as LNA™ GapmeRs) which catalyze RNaseH-dependent degradation of both mRNAs and lncRNA. LNA™ GapmeRs have been tested to show knockdown of multiple classes of RNA targets in vitro, including mRNA and lncRNA targets with both nuclear and cytoplasmic subcellular localizations. Our results demonstrate that these targets were equally efficiently silenced by our Antisense LNA™ GapmeRs with a good hit rate irrespective of the type of RNA target and its subcellular localization. In vivo, we report highly efficient and long lasting knockdown of a nuclear retained lncRNA in a broad range of tissues in mice subjected to systemic administration of a LNA™ GapmeR.

To design LNA™ GapmeRs, we have developed an empirically-derived design algorithm to provide ASOs that achieve potent target knockdown with a high hit-rate. A recent publication[1] and our own preliminary results indicate that unspliced primary transcripts are in fact the true targets of gapmers and therefore the design of LNA™ GapmeRs targeting introns is a valid and efficient approach to preventing the production of mature spliced transcripts. However, these results have further implications for the design to avoid off-targets, which are a relevant concern for all antisense strategies. To address potential off-targets located in either exons or introns, our LNA™ GapmeR design algorithm searches both spliced and unspliced transcriptomes in the Ensemble database, to provide maximal target specificity while cytotoxicity will be added later as an additional contributing parameter to the design algorithm.

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High-Risk Plasma microRNA Signature Is Associated with an Immune-Related Gene Expression Profile of Lung Tumour Tissues

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MicroRNAs (miRNAs) are short non-coding RNAs regulating gene expression. We have previously validated a plasmatic miRNA-signature classifier (MSC) with diagnostic and prognostic value in lung cancer LDCT-screening programs. Here, we investigated the association between MSC risk level and corresponding tumour gene expression profile.

Lung tumour tissues collected from 19 patients of a pilot screening trial with an available plasma MSC result were profiled for microarray gene expression. By class comparison analysis, a signature of 238 genes was associated with plasma MSC risk level. Genes included in the signature were mainly involved in processes associated with lung cancer aggressiveness, particularly in pathways affecting the host immune response.

The support vector machine (SVM) supervised learning model was then used to validate the tumour tissue gene signature in 21 lung cancer patients of an independent screening trial. The SVM classified patients according to MSC with a sensitivity and specificity of 0.9 and 0.73 (P=0.01). In addition, by in situ hybridization, some of the miRNAs composing the MSC were found differentially expressed between tumour infiltrating lymphocytes and their normal counterpart. Overall, these findings suggest that a circulating miRNA-based risk level may reflect an impaired gene expression profile in the tumour interacting with the immune system.

Genome-Wide miRNA Analysis Identifies miR-188-3p as a Novel Prognostic Marker and Molecular Factor Involved in Colorectal Carcinogenesis.

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Purpose: Characterization of colorectal cancer transcriptome by high-throughput techniques has enabled the discovery of several differentially expressed genes involving previously unreported miRNA abnormalities. Here, we followed a systematic approach on a global scale to identify miRNAs as clinical outcome predictors and further validated them in the clinical and experimental setting. Experimental Design: Genome-wide miRNA sequencing data of 228 colorectal cancer patients from The Cancer Genome Atlas dataset were analyzed as a screening cohort to identify miRNAs significantly associated with survival according to stringent prespecified criteria. A panel of six miRNAs was further validated for their prognostic utility in a large independent validation cohort (n = 332). In situ hybridization and functional experiments in a panel of colorectal cancer cell lines and xenografts further clarified the role of clinically relevant miRNAs. Results: Six miRNAs (miR-92b-3p, miR-188-3p, miR-221-5p, miR-331-3p, miR-425-3p, and miR-497-5p) were identified as strong predictors of survival in the screening cohort. High miR-188-3p expression proves to be an independent prognostic factor [screening cohort: HR = 4.137; 95% confidence interval (CI), 1.568–10.917; P = 0.004; validation cohort: HR = 1.538; 95% CI, 1.107–2.137; P = 0.010, respectively]. Forced miR-188-3p expression increased migratory behavior of colorectal cancer cells in vitro and metastases formation in vivo (P < 0.05). The promigratory role of miR-188-3p is mediated by direct interaction with MLLT4, a novel identified player involved in colorectal cancer cell migration. Conclusions: miR-188-3p is a novel independent prognostic factor in colorectal cancer patients, which can be partly explained by its effect on MLLT4 expression and migration of cancer cells.

Functional long Noncoding RNAs in Cancer Pathways

Maite Huarte Martinez

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A major shift in our conception of genome regulation has emerged in recent years. It is now obvious that the majority of cellular transcripts do not code for proteins, and a significant subset of them are long RNAs (lncRNAs). Moreover many lncRNAs have been shown to be functional, and are emerging as important regulatory molecules in tumor-suppressor and oncogenic pathways. Supporting this idea, we found that the transcription factor p53, which is crucial for the maintenance of cellular homeostasis, specifically regulates the expression of dozens of lncRNAs, and constitute active components of this important tumour suppressor pathway. In contrast, other lncRNAs can promote the malignant phenotype of cancer cells, acting as oncogenes. We will present our findings implicating lncRNAs in the regulation of the transformed phenotype of cancer cells, with particular attention to the molecular mechanisms by which they affect gene function.

Plasma Viral miRNAs as Targeted-biomarkers of Occult Viral Infections Prevalence and Sepsis Aggressiveness

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Prevalence of Kaposi sarcoma-associated herpesvirus (KSHV/HHV-8) varies greatly in different populations. We hypothesized that the actual prevalence of KSHV/HHV8 infection in humans is underestimated by the currently available serological tests. We analyzed four independent patient cohorts with post-surgical or post-chemotherapy sepsis, chronic lymphocytic leukemia and post-surgical patients with abdominal surgical interventions. Levels of specific KSHV-encoded miRNAs were measured by reverse transcription-quantitative polymerase chain reaction (RT-qPCR), and KSHV/HHV-8 IgG were measured by immunoassay. We also measured specific miRNAs from Epstein Barr Virus (EBV), a virus closely related to KSHV/HHV-8, and determined the EBV serological status by ELISA for Epstein-Barr nuclear antigen 1 (EBNA-1) IgG. Finally, we identified the viral miRNAs by *in situ* hybridization (ISH) in bone marrow cells. In training/validation settings using independent multi-institutional cohorts of 300 plasma samples, we identified in 78.50% of the samples detectable expression of at least one of the three tested KSHV-miRNAs by RT-qPCR, while only 27.57% of samples were found to be seropositive for KSHV/HHV-8 IgG ($P< 0.001$). The prevalence of KSHV infection based on miRNAs qPCR is significantly higher than the prevalence determined by seropositivity, and this is more obvious for immuno-depressed patients. Plasma viral miRNAs quantification proved that EBV infection is ubiquitous. Interestingly, we showed that KSHV-miRNAs are implicated in sepsis and may drive enhanced secretion of pro-inflammatory and anti-inflammatory cytokines exacerbating sepsis. Since chronic viral infections represent risk factors for diseases and development of infection-related complications, measurement of viral miRNAs by qPCR has the potential to become the “gold” standard method to detect certain viral infections in clinical practice, especially in patients with low number of immune cells.

Stepwise Analysis of MIR9 Loci Identifies miR-9-5p to Be Involved in Oestrogen Regulated Pathways in Breast Cancer Patients

Raffaela Barbano[1], Barbara Pasculli[1], Rendina Michelina[1], Andrea Fontana[2], Caterina Fusilli[3], Massimiliano Copetti[2], Stefano Castellana[3], Vanna Maria Valori[4], Paolo Graziano[5], Michelina Coco[1], Francesco Picardo[6], Tommaso Mazza[3], Ella Evron[7], Evaristo Maiello[4], Manel Esteller[8], Vito Michele Fazio[1], Paola Parrella[1]

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miR-9 was initially identified as an epigenetically regulated miRNA in tumours, but inconsistent findings have been reported so far. We analysed the expression of miR-9-5p, miR-9-3p, pri-miRs and the methylation status of MIR9 promoters in 131 breast cancer cases and 12 normal breast tissues (NBTs). The expression of both mature miRs was increased in tumours as compared to NBTs ($P < 0.001$) and negatively correlated with ER protein expression ($P = 0.005$ and $P = 0.003$, for miR-9-3p and miR-9-5p respectively). In addition, miR-9-5p showed a significant negative correlation with PgR ($P = 0.002$). Consistently, miR-9-5p and miR-9 3p were differentially expressed in the breast cancer subgroups identified by ER and PgR expression and HER2 amplification. No significant correlation between promoter methylation and pri-miRNAs expressions was found either in tumours or in NBTs. In the Luminal breast cancer subtype, the expression of miR-9-5p was associated with a worse prognosis in both univariable and multivariable analyses. Ingenuity Pathway Analysis exploring the putative interactions among miR-9-5p/miR-9-3p, ER and PgR upstream and downstream regulators suggested a regulatory loop by which miR-9-5p but not miR-9-3p is induced by the steroid hormone receptor and acts within hormone-receptor regulated pathways.

4
Poster Presentation Abstracts

1 CircRNA Characterisation in Breast Cancer Cells

Lucia Coscujuela Tarrero[1], Giulio Ferrero[2], Valentina Miano[1], Laura Ricci[1], Raffaele Calogero[3], Maddalena Arigoni[3], Federica Riccardo[3], Marco Beccuti[2], Carlo De Intinis[2], Francesca Cordero[2], Michele De Bortoli[1]

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Circular RNAs (circRNAs) are a class of endogenous, stable RNAs originated from the back-splicing of internal gene exons. Human breast cancer cells of the luminal subtype are strongly dependent on Estrogen Receptor α (ERα), which regulates the transcription of both coding and non-coding luminal-specific genes. While abundant data is available on the transcriptome of the main cell model of luminal BC, the MCF7 cell line, information on circRNA is still lacking. We performed paired-end poly(A-) RNA-Seq analysis of MCF-7 cells grown in four different culture conditions, each in triplicate, in order to obtain an as wide as possible landscape from these cells. Using the CIRI algorithm, we predicted 3,271 circRNAs. To characterize in more detail the genomic properties of circRNA host genes, we developed a novel computational tool (*CircHunter).* The analysis of circRNA host genes confirmed that circularization involves preferentially internal exons at the 5' end of host gene body, most frequently the second and the third. We also could confirm that genes hosting circRNA are longer, give rise to a higher number of transcripts and have longer first introns, when compared to control and random gene sets. Using qRT-PCR and *RNseR* treatment, we could validate 28 out of thirthy circRNAs of this set. We present here data on their expression level in a series of breast cancer cell lines. Furthermore, through the analysis of public ChIP-Seq experiments and experimental validation in MCF7 cells, we discovered that circRNA host genes are significantly enriched in H3k36me3 as compared to control genes producing only linear transcripts. Notably, this histone mark displays a very high signal at the 5' exons involved in back-splicing. In order to analyse circRNA in public total RNA-Seq datasets from tumor tissues, we developed an alignment-free method to direct compare sequencing reads with reconstructed back-splice sequences. The back-spliced sequences of our 3,271 circRNAs were examined in public datasets of triple negative, ER positive, HER2 amplified and normal tissues. 113 circRNAs were found to be differentially expressed between Triple Negative and ER+ tumours and 622 circRNAs differentially expressed between ER+ and normal tissue. We discovered interesting candidates that could have a miRNA sponge function. Knock-down experiments using siRNA specific for the back-splicing junction of these circRNA will unravel their role as post-transcriptional regulators in breast cancer cells.

2 Circular RNA in Cancer

Marjan E. Askarian-Amiri[1], Debina Sarkar[1], Herah Hansji[1], Kaveesha Bodiyabadu[1], Euphemia Y. Leung[1], Stefan K. Bohlander[2], Graeme J. Finlay[2], Bruce C. Baguley[3]

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Circular RNAs (circRNAs) are covalently closed transcripts in which a downstream 3' splice donor site fuses with an upstream 5' acceptor site. We have shown that the SRY-box2 (SOX2) mRNA forms a circRNA. CircSOX2 is derived from the 3' UTR of SOX2 and is localised in the nucleus. We have also shown that SOX2OT, an overlapping transcript of SOX2, forms a circRNA and like linear SOX2OT, is localised in the nucleus. The expression levels of the circular and linear SOX2OT transcripts are not correlated, whereas linear SOX2 and SOX2OT expression is strongly correlated. We also found that both linear and circular SOX2OT are upregulated following drug treatment.We have also shown that the lncRNA ZFAS1 forms a novel circular RNA in a breast cancer cell line. Different isoforms of circZFAS1, such as linear ZFAS1, bound to monosomes and light polysomes.In melanoma cell lines, we have identified fifty different isoforms of circRNAs processed from ANRIL, and have shown that they are predominantly localised in the cytoplasm while linear ANRIL is located in the nucleus. This suggests two distinct functions for these RNA species. Our data suggest that linear and circular RNA derived from the same gene may have distinct regulatory roles in cancer cells.

3 Deregulation of Long Non-coding RNA WHSC2-2 in Multiple Myeloma

Cristina Vinci[1, 2], Elisa Taiana[1, 2], Domenica Ronchetti[1, 2], Luca Agnelli[1, 2], Martina Manzoni[1, 2], Serena Galletti[1, 2], Marta Lionetti[1, 2], Katia Todoerti[3], Antonino Neri[1, 2]

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Recent studies have demonstrated the involvement of long non-coding RNAs (lncRNAs) in gene expression, cell biology and carcinogenesis. Our laboratory investigated the expression of lncRNAs in multiple myeloma (MM), a malignant proliferation of antibody-secreting bone marrow plasma cells (PCs). Specifically, we developed a custom annotation pipeline of GeneChip® Human Gene 1.0 ST microarray data, able to investigate more than 1800 lncRNAs. The analyses of highly purified bone marrow PCs from 170 MM primary tumors and nine normal donors led to the identification of 31 lncRNAs specifically deregulated in MM. In particular, lnc-WHSC2-2, a pseudogene located in the intron 19 of the *MMSET* gene, was significantly upregulated in MM patients carrying t(4;14) chromosomal translocation, which specifically deregulates *MMSET*. In human multiple myeloma cell lines (HMCLs), lnc-WHSC2-2 displayed a nuclear subcellular localization and a very short half-life. In MM samples, lnc-WHSC2-2 and *MMSET* expression levels turned out to be highly correlated. In addition, siRNA silencing of MMSET in HMCLs caused a time-dependent down-regulation of lnc-WHSC2-2, suggesting a co-regulation mechanism. Finally, HMCLs treated with SAHA showed lnc-WHSC2-2 transcriptional repression, suggesting that it might undergo epigenetic regulation.
These preliminary results led us to hypothesize that lnc-WHSC2-2 could have a role in transcriptional regulation and could be involved in the pathogenesis of MM disease.

4 EWSR1 in the Maintenance of Genomic Stability

Stefanie Berger

University of Basel, Switzerland.

EWSR1 was discovered in Ewing Sarcomas, where, following chromosomal translocations, it forms EWSR1-fusion proteins with oncogenic activity. While many studies have addressed the function of these fusion proteins, information concerning EWSR1's natural role is sparse.
We showed that transient knock-down induces an increase in DNA double strand breaks, instability of its own locus at the site where breaks most often occur in Ewing Sarcoma, and cellular lethality.
To avoid transient transfections, we established Flp-In TREx HeLa cells allowing inducible knock-down of EWSR1 to examine the effects of EWSR1 depletion on the occurrence of DNA damage. Upon knock-down of EWSR1, we detect more DNA damage in comet assays, an increase of 53BP1 foci in immunostainings, and an upregulation of γH2AX by Western Blots. While cell cycle profiles appear undisturbed, less mitotic cells could be observed in the knock-down population. However, knock-down efficiencies vary and are heterogeneous across the population. This incomplete knock-down might explain why the observed effects are relatively weak.
With an adapted knock-out approach we will examine the influence of EWSR1 depletion on the maintenance of genomic integrity and aim on revealing the underlying mechanisms such as binding to R-loops, transcriptional progression, DNA synthesis rate, and mitosis.

5 Expression and Functional Characterization of Ultraconserved Non-Coding Regions 8+ in Bladder Cancer

Sara Terreri[1], Sara Mancinelli[1], Dimitrios Papaioannou[2], Giovanna L. Liguori[1], Ramiro Garzon[2], Amelia Cimmino[1]

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Transcribed ultraconserved regions (T-UCRs) represent a group of highly conserved sequences among orthologous regions of human, rat and mouse genomes. They represent a new class of long non-coding RNAs (lncRNAs) whose function is still unknown. While microRNAs and other types of lncRNAs have been shown to contribute to the biological function of bladder cancer (BlCa) and are increasingly being used to improve the clinical care of patients, this is not yet the case for T-UCRs. By using genome-wide profiling, we identified 293 T-UCRs de-regulated in BlCa patients as compared to normal tissues. T-UCR 8+ is the most up-regulated and inversely related to grade, paving the way for clinical applications. We demonstrated that T-UCR 8+ is localized in the cytoplasm of BlCa cells, suggesting that active molecular exportation could take place and be involved in cancer formation and/or progression. To better clarify the cytoplasmic function of T-UCR 8+ during tumorigenesis and understand its role, we dissect T-UCR 8+ protein network interaction using the RAP–MS method. As preliminary data, we purified T-UCR 8+ sequence and we are now performing the mass spectroscopy to verify which proteins bind to this lncRNA. To confirm its localization, we also performed ISH (*In situ* Hybridization) experiments in BlCa tissues and different embryo stages.

6 Luminal lncRNAs Regulation by ERα-controlled Enhancers in a Ligand-independent Manner in Breast Cancer Cells

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Estrogen receptor-α (ERα) is a ligand-inducible protein which mediates estrogenic hormones signaling and defines the luminal breast cancer (BC) phenotype. Recently, we demonstrated that ERα binds chromatin in absence of ligand (apoERα) regulating transcription of protein-coding genes and several lncRNAs. Noteworthy, apoERα-regulated lncRNAs marginally overlap estrogen-induced transcripts representing a signature of luminal BC genes. DSCAM-AS1 is a paradigmatic example of apoERα activity since its expression is largely unaffected by estrogenic treatment despite an E2-induced increment of ERα binding on its promoter.

Analysing H3K27ac ChIP-Seq performed in hormone-deprived MCF-7, we identified a set of Super Enhancers (SEs) occupied by apoERα including one mapped in proximity of DSCAM-AS1. Using ChIP-qPCR, we validated ChIP-Seq signal of apoERα, p300 and CTCF at both DSCAM-AS1 TSS and at its associated SE. Furthermore, analysing MCF-7 ChIA-PET data and performing a 3C experiment, we confirmed a long range chromatin interaction between the SE and the DSCAM-AS1 TSS. Interestingly, CTCF binding downstream to DSCAM-AS1 shows an enrichment in hormone-depleted medium as compared to other experimental conditions, indicating that CTCF demarcates enhancer actions at DSCAM-AS1 locus.

The analysis of this lncRNA provides a paradigm of transcriptional regulation of a luminal specific apoERα regulated lncRNA.

7 miR-612 Suppresses Cancer Stem Cell-like Property of Hepatocellular Carcinoma by Sp1/Nanog Signaling

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In our previous study we found that miR-612 negatively regulated stem cell-like property and tumor metastasis of HCC. In this study, we try to elucidate underlying regulation mechanism , and find that miR-612 inversely modulate the mRNA and protein level of EpCAM as well as CD133, negatively regulate the numbers and sizes of tumor spheres, directly inhibit the protein level of Sp1, and subsequently reduce transcription activity of Nanog. Of importance, the higher levels of Sp1 and Nanog in biopsies are the more unfavorable prognoses of HCC patients after tumor resection. Taken together, miR-612 plays a suppressive role on HCC stemness via Sp1/Nanog signaling pathway.

8 The Therapeutic Potential of Small Molecules in the Treatment of Cancer Cachexia

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Despite advancement in pharmaceutical therapies, cancer is still the leading cause of death in the world. In fact, nearly one-third of cancer death can be attributed to cachexia that is a dysregulated metabolic state characterized by a progressive loss of skeletal muscle mass. It can be extremely debilitating and correlates with poor quality of life and a high mortality rate in cancer patients. Drugs that can prevent muscle loss would be a solution, but there is currently no remedy available for clinical use. We recently found that bexarotene, a clinically approved selective ligand of the retinoid X receptors (RXRs), promotes the differentiation and fusion of skeletal myoblasts through the activation of RXRs as a transcription factor. We also found that bexarotene is able to counter the detrimental effects of tumor-derived factors and retains myogenic differentiation following pro-atrophic insult. More importantly, we have identified an isoform-specific role for Akt in RXR-selective signaling to promote and retain myogenesis. This new model of rexinoid-enhanced myogenesis provides an excellent avenue to identify additional genetic targets and molecular interactions for delineating the molecular mechanisms of myogenesis and for therapeutic development towards muscle regeneration. We thus conducted integral RNA-seq and ChIP-seq analyses to profile molecular pathways associated with rexinoids during myoblast differentiation. Our goal is to provide fundamental insights and enable the rational development of a clinically translatable protocol to exploit multiple signaling pathways for promoting muscle regeneration with the potential to facilitate the prevention and treatment of cancer cachexia.

9 Upregulated lncRNA-HNGA1, a Target of miR-378a, Contributes to Aerobic Glycolysis of Head and Neck Squamous Cell Carcinoma Throughincreasing Levels of the C-C Chemokine Receptor Type 7 (CCR7)

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Introduction: Long non-coding RNAs (lncRNAs) have been regarded as key regulators in aerobic glycolysis of human cancer. However, therole and function of lncRNAs in Head and Neck Squamous Cell Carcinoma (HNSCC) aerobic glycolysis remain unclear. Here, we report a novel lncRNA, HNGA1, which could promote HNSCC aerobic glycolysis and malignancy by competing for miR-378a binding to regulate CCR7.

Materials and methods: Microarrays were performed to explore the lncRNA/miRNA profiles in tissues samples. qRT-PCR and functional analysis were used to confirm the expression and role of lncRNA/miRNA. Bioinformatics approach and luciferase assay were used to verify the miRNA target gene and the interation between lncRNA and miRNA. Nude mouse model was utilized to observe the effect of lncRNA/miRNA in vivo. Tissue array was performed to explore the association between lncRNA and postoperative survival.

Results:

1.LncRNA HNSCC glycolysis-associated 1 (HNGA1) was up-regulated in tumor tissues, while miR-378a was down-regulated significantly. These
observations were confirmed in 60 pairs of HNSCC tissues/non-tumor tissues samples and 7 cohorts of HNSCC cell lines.

2. Silencing ofHNGA1 inhibited HNSCC cells proliferation and glycolysis, while overexpression of HNGA1 had the opposite effect.

3. Ectopic expression ofmiR-378a repressed HNSCC cells proliferation and glycolysis, whereas miR-378a inhibition resulted in the opposite effect. MiR-378a couldrepress the CCR7 expression by binding to the 3'-UTR region of CCR7 directly.

4. There was an inverse correlation between HNGA1 and miR-378a in HNSCC specimens. Moreover, miR-378a suppressed HNGA1's expression and function by directly binding to HNGA1. In addition,HNGA1 could reverse the inhibitory effect of miR-378a on HNSCC cells, which might act as an endogenous 'sponge' by competing for miR-378a binding to regulate CCR7.

5. The xenograft mouse model unveiled the suppressive effects of miR-378a on HNSCC tumor growth and glycolysis, while HNGA1 could accelerate this process.

6. The clinicopathological findings suggested that the up-regulation of HNGA1 in HNSCC patients was associated with the poorly differentiated degree and more metastasis. Moreover, the results of tissue array showed that HNGA1 was correlated with postoperative survival.

Conclusion: Taken together, our data highlights the pivotal role of HNGA1 in HNSCC aerobic glycolysis. More importantly, we elucidate a novel lncRNA-miRNA-mRNA regulatory network that is HNGA1-miR-378a-CCR7 axis in HNSCC malignancy and progression.

10 Differentially Expressed lncRNA Transcripts in Clinical Subtypes of Breast Carcinoma

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Long noncoding RNAs (lncRNAs) are unfolding as a class of RNA transcripts involved in crucial biological processes from development to disease progression. Several lncRNAs have already been established to play a role in tumorigenisis, but the lncRNA repertoire in specific cancers is still expanding. New methods including transcriptome sequencing have improved and continue to improve discovery and quantification of novel and known lncRNA transcripts. To identify lncRNAs expressed in human breast carcinoma, as well as lncRNAs associated to clinical subtypes of breast cancer, we applied ab initio transcript assembly to RNA-sequencing (RNA-seq) from 35 breast cancer patients from The Cancer Genome Atlas (TCGA) Breast Carcinoma (BRCA) cohort, as well as 40 samples from an independent patient cohort (Radium/Rutgers). A number of previously annotated lncRNAs were identified in both the TCGA and Rutgers/Radium patient samples. Among these, both previously known as well as novel lncRNAs were identified with differential expression among Estrogen Receptor (ER) positive and ER negative tumors. In addition to well known lncRNAs such as *GAS5* and *H19*, we identified a number of novel lncRNAs with differential expression between the two clinical subtypes.

11 Long noncoding RNA PVT1 as Promising Biomarker for Colorectal Adenocarcinoma

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Long noncoding RNAs (lncRNAs) are lengthy noncoding RNAs which are involved in critical signaling pathways like cell cycle and apoptosis, it is not surprising to see their altered expressions in human tumors. Colorectal adenocarcinoma is one the most frequent malignancies worldwide. The role of lncRNAs in colorectal adenocarcinoma is not well understood. To study the significance of lncRNAs in colorectal adenocarcinoma, we retrieved 189 approved lncRNAs from HGNC. The genes were imported into the cBioPortal database for transcriptomic analyses. We queried all the samples from TCGA provisional colorectal adenocarcinoma with RNA-seq v2 data (n=379) in our study and considered RNA dysregulation with Z-score threshold: ±2. The lncRNA which was altered in most of the patients were considered as "significant lncRNA" for further analyses. We also extract the genes which were co-expressed with candidate lncRNA using Pearson's correlation >0.50. The expressed genes uploaded into the GSEA dataset to compute the gene set overlaps matrix based on the GO molecular function. Our analysis showed that lncRNAs PVT1 allocated the maximum alteration among the Colorectal adenocarcinoma cases. We also found that overall survival has been reduced in patients with dysregulation for PVT1 (Logrank Test p-Value= 0.05). Additionally, we found 45 genes on 8q22-gq24 which were coexpressed with PVT1 showing the importance of this region on chromosome 8 in colorectal adenocarcinoma pathogenesis and some of them non-covalently interact with PolyA in transcripts. Altogether these data showed that 8q-associated genes especially PVT1 are promising candidates for colorectal adenocarcinoma biomarker discovery although more experimental works are needed to confirm.

12 Long-Non Coding RNA Deregulation in Non-Small Cell Lung Cancer

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Long-non coding RNAs (lncRNAs) have been shown to regulate numerous biological processes and diseases such as human cancer. While the list of deregulated lncRNAs in cancer is growing, the mechanism of this deregulation still needs to be elucidated.

Following a lncRNA microarray screening in 44 pairs of NSCLC specimens, we validated by qPCR the level of deregulation in 67 independent pairs of lung tumour and normal tissues. We selected four validated lncRNAs (NUTM2A-AS1, FEZF1-AS1, LINC01214, and LINC00673) and investigated the potential epigenetic regulation of their expression. NUTM2A-AS1 and FEZF1-AS1, bearing CpG islands in their promoters were examined by pyrosequencing for DNA methylation changes in primary NSCLC tissues. Only FEZF1-AS1 demonstrated hypermethylation. In addition, the expression of all four lncRNAs was investigated in NSCLC cell lines, in the presence of a DNA methylation and histone deacetylase inhibitor (decitabine and valproic acid respectively). NUTM2A-AS1 showed no differences, while the expression of FEZF1-AS1, LINC01214, and LINC00673 was modified by these epigenetic drugs.

In conclusion, epigenetic modulation of lncRNAs is one of the important reasons for their deregulation in NSCLC, in a direct or indirect manner.

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13 Potential Role of non-coding RNA in Hepatocellular Carcinoma Revealed by Whole Exome Sequencing

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The dysfunction of non-coding RNA (ncRNA) within the body can cause a variety of diseases. Having a better understanding of global mutation pattern in non-coding RNA has tremendous potential to advance our understanding of cell regulatory and disease mechanisms. The whole exome sequencing of multiple cell lines was done by Hiseq 2500. in multiple cell lines (Bel-7402, Bel-7404, SMMC-7721, HepG2, Hep3B, MHCC-97H, MHC-97L). We identified 421 SNP and 97 indel mutation sites in the ncRNA exonic region from these 7 cell lines. Among these mutations, 21 SNP mutation in 20 ncRNA (SCARNA18, PCDHGB8P, CMAHP, LINC00336, LOC100132891, LOC100652768, LINC00346, LOC100129794, LOC100129345, MIR2392, LOC253044, PRSS30P, LOC100130950, LOC100287072, LOC440461, LOC388499, NAPSB, PRNT, MIR4326, XIST) and 12 indel mutation in 10 ncRNA (LGALS8-AS1, PCDHB18, MALAT1, KIRREL3-AS3, MKRN9P, PAN3-AS1, C14orf23, SSTR5-AS1, SENP3-EIF4A1, LINC00469) were generally existed in all the 7 cell lines, also have low incidence in health condition as compared to the data in 1000 Genomes Project. The in silico analysis showed that these mutation may play pivotal role in the development of HCC. This indicated that these mutation of ncRNA has the potential for being biomarkers in HCC, which still need to be validate in the patient sample and peripheral blood.

14 Deciphering Post-transcriptional Gene Regulatory Networks Sustaining Sprouting Angiogenesis

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Current strategies to tackle angiogenesis are largely based on reductionist studies mainly designed to identify the role of ligand/receptor pairs. However, the complexity of the angiogenic program needs new approaches to decipher the properties of the signaling networks within the cells. Sprouting angiogenesis (SA) is a multistep process that requires in its different phases an orchestrated control of many cellular functions: activation of quiescent endothelial cells (ECs) by an angiogenic inducer, basal lamina and extracellular matrix degradation, cell migration, and cell proliferation. This is accomplished through the activity of multiple layers of regulation both at transcriptional and post-transcriptional level. However, the extent to which microRNAs activity globally affects all the biological pathways sustaining SA has not been explored so far. To fully capture the phenotypic changes occurring to endothelial cells during SA in its complexity and to measure the impact of microRNAs activity, we exploited a 3D model of SA that recapitulates *in vitro* the angiogenic program. In this model, human umbilical vein ECs (HUVECs) are induced to form 3D aggregates, or spheroids, to mimic the endothelial quiescent state. Spheroids are then embedded in a collagen matrix and exposed to VEGF-A to trigger the angiogenic sprouting. Samples from quiescent or stimulated spheroids were subjected to RNA-Sequencing of coding and non-coding RNAs. Expression data were validated by functional assays. To estimate the impact of microRNAs activity we developed a specific bioinformatics pipeline that includes the co-expression analysis between protein-coding genes and microRNAs expressed in our dataset, and the prediction of microRNA interactions. When considering only pairs containing predicted microRNA-target gene interactions we observed a significant enrichment in negative correlations, indicating that expression analysis combined with prediction of microRNA target genes is able to identify functional interactions that more likely are affected by microRNAs activity. This allowed the mapping of the global post-transcriptional regulatory network that sustains SA. Analysis of this network revealed a scale-free topology. Interestingly, several microRNAs showed preferential targeting activity towards genes associated to specific cellular functions involved in SA, such as cell cycle and cell migration. This study suggests that microRNAs have a profound impact on the biological pathways relevant to SA, and play an important role in the switch from quiescent to activated EC. Functional validations by gain of function and loss of function of hub microRNAs together with validation of expression in tissues from colorectal cancer patients are currently ongoing.

15 Expression Profiling Identifies lncRNA HNRNPU-AS1 With Oncogenic Properties In Pancreatic Ductal Adencocarcinoma

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A gene array was used to profile the expression of > 22,000 lncRNAs and > 33,000 protein coding genes in 48 specimens of pancreatic ductal adenocarcinoma (PDAC), adjacent benign pancreas and the pancreas from patients without pancreatic disease. Of the lncRNAs profiled, the expression of 414 were significantly increased and 179 were decreased in the tumors ($p<0.05$, 2-fold change). The expression of the lncRNA HNRNPU-AS1 was among the most significantly deregulated (increased 8-fold) in the tumors compared to normal/adjacent benign tissues. Increased expression of HNRNPU-AS1 was associated with poor prognosis for patients with pancreatic ductal adenocarcinoma. Increased expression of HNRNPU-AS1 was observed in PDAC cell lines compared to noncancerous pancreatic cell lines. LNA gapmer mediated inhibition of HNRNPU-AS1 reduced cell proliferation in Patu-T and PL45 pancreatic cancer cell-lines. Reduced invasion and migration was also reported upon lncRNA knockdown in Patu-T cells. Mechanistic studies further exhibited that reducing lncRNA expression effectively reduced both TGFRB2 mRNA and protein expression in Patu-T cells. Small RNA sequencing of the gapmer treated and control cells established that miR-1246 was increased by 7-fold upon knockdown of HNRNPU-AS1. A portion of the HNRNPU-AS1 transcript is predicted to bind to the 7 nt seed region of miR-1246 and miR-1246 is predicted to target TGFBR2 mRNA suggesting that HNRNPU-AS1 functions as a competing endogenous RNA for miR-1246. The lncRNA HNRNPU-AS1 expression is increased in PDAC tissues and cell lines and HNRNPU-AS1 promotes proliferation and invasion/migration in part due to regulation of TGFBR2.

16 MicroRNA-196 Influence Metastases Formation in Colorectal Cancer through Regulation of HOXB and GALNT Gene Expression

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MicroRNA-196 has previously been implicated in malignant transformation, however its role in colorectal cancer (CRC) has not yet been fully explored. In the current study, we examined the clinical and biological relevance of miR-196, and the molecular pathways regulated by miR-196 in CRC.MiR-196 expression were quantitated by qRT-PCR in two independent cohorts comprised of 292 CRC patients in total, to explore its biomarker potential. Transient and stable gain and loss of function experiments were conducted in a panel of CRC cell lines, to evaluate the impact of miR-196 on proliferation, chemo-sensitivity, migration/invasion and metastases formation in vitro and in vivo. The molecular pathways influenced by miR-196 were characterized using whole transcriptome profiling, in-silico target prediction tools, luciferase-interaction assays, and pheno-copy gene knock-down experiments. Low miR-196 expression was significantly associated with metastatic status and poor outcomes in both independent CRC patient cohorts ($p<0.05$, log-rank test). MiR-196 suppression led to increased CRC cell migration/invasion and metastases formation in mice, whereas ectopic overexpression showed the opposite phenotype. Molecular profiling and target confirmation identified an interaction between miR-196 and *HOXB* and *GalNT,* which in turn regulates CRC cell migration. Our findings provide new insights into the role of miR-196 in CRC metastases formation through regulation of proteins involved in CRC progression.

17 Loss of miR-449a in ERG-Associated Prostate Cancer Promotes the Invasive Phenotype by Inducing SIRT1

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Epigenetic regulation by SIRT1, a multifaceted NAD^+-dependent protein deacetylase, is one of the most common factors modulating cellular processes in a broad range of diseases, including prostate cancer (CaP). SIRT1 is over-expressed in CaP cells, however the associated mechanism is not well understood. To identify whether specific microRNAs might mediate this linkage, we have screened a miRNA library for differential expression in ERG-associated CaP tissues. Of 20 differentially and significantly expressed miRNAs that distinguish ERG-positive tumors from ERG-negative tumors, we find miR-449a is highly suppressed in ERG-positive tumors. We establish that SIRT1 is a direct target of miR-449a and is also induced by ERG in ERG-associated CaP. Our data suggest that attenuation of miR-449a promotes the invasive phenotype of the ERG positive CaP in part by inducing the expression of SIRT1 in prostate cancer cells. Furthermore, we also find that suppression of SIRT1 results in a significant reduction in ERG expression in ERG-positive CaP cells, indicating a feed-back regulatory loop associated with ERG, miR-449a and SIRT1. We also report that ERG suppresses p53 acetylation perhaps through miR-449a-SIRT1 axis in CaP cells. Our findings provide new insight into the function of miRNAs in regulating ERG-associated CaP. Thus, miR-449a activation or SIRT1 suppression may represent new therapeutic opportunity for ERG-associated CaP.

18 SLN-based Drug Delivery Systems to Overcome Drug Resistance in Breast Cancer

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Since their introduction in 1991, **solid lipid nanoparticles (SLN)** have emerged as potential carriers for poorly-soluble drugs. Such nanoparticle-based drug delivery strategies seem to effectively enhance the targeted delivery of therapeutics to tumors and overcome the side effect(s) of chemotherapy. Indeed, SLN possess a reactive surface that can be easily modified with biocompatible coatings/shell before loading the lipid core as well as the shell with therapeutic agents, including miRNA, siRNA and anti-cancer drugs, for the post-transcriptional regulation of *malignant* growth, differentiation, apoptosis, motility, and transformation. In this work, we aim to investigate the potential of modified solid lipid nanoparticles for the controlled (and/or metered) delivery of paclitaxel (PAX). SLN loaded with PAX were prepared via modified high-pressure hot homogenization. Formulation parameters were optimized to obtain a high-quality delivery system. SLN cores were coated, layer-by-layer, with a chitosan and hyaluronan (HA) shell. Selectivity toward HA receptors was tested in a breast cancer cell line, MCF-7. Here in, the design, formulation and physico-chemical characterization of stable and reproducible nano-sized and negatively charged **core-shell nanocapsules** are presented. Findings reveal that chitosan-HA-coated SLN facilitated the targeting, cellular uptake and the time-/dose-controlled delivery and release of PAX, enhancing intrinsic chemotherapeutic activities. We conclude that SLN are suitable carrier candidates for nano-oncology given their localized, and potent cytotoxic potential overcoming multidrug-resistant cancer cells.

19 Identification of Metabolites Influencing Multiple Sclerosis Development and Metabolites Associated with Common Clinical Symptoms Using Quantitative Analysis of Magnetic Resonance Imaging

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Introduction: Multiple sclerosis (MS) is an autoimmune disease that affects the central nervous system. To determine the pathology of the disease— in some cases, to distinguish between MS plaque and other pathological lesions (such as tumors, edema)—and also because of the unavailability of brain lesions, another method besides biopsy should be used. Magnetic resonance spectroscopy is an analytical non-invasive method to obtain pathological information about the disease and provide biochemical information about the studied tissue that can be useful when investigating the causes and progression of the disease and increase the detection sensitivity for Neurologists. In this study, we try to examine the ability of this method to display the early and differential diagnosis of subgroups of multiple sclerosis patients.

Methods: In this study, 45 patients with multiple sclerosis in three subgroups; RRMS (12 women and four men) PPMS (seven women and eight men) and SPMS (nine women and six men) and 16 healthy subjects as controls (10 women and six men) participated. MRI protocols were performed for each patient, then the MRS protocol was done for the two regions of interest (VOI) of the brain. In the one stage, VOI are selected from within the plaque area and others are selected within the normal appearing white matter (NAWM). Using statistical tests, the metabolites were evaluated in three sub-groups and the amount of metabolites in plaque and NAWM in each subgroup of patients was compared. By categorizing patients with four common clinical symptoms such as blurred vision, movement disorders or muscle weakness, loss of balance and sensory disorders in certain groups, the correlation of these symptoms with metabolites was determined.

Results: Metabolite of Cr(Cratean) in the NAWM between SPMS and RRMS subgroups and between PPMS and RRMS, respectively, ($P = 0.001$ and $P = 0.03$) was significantly different. Cho was significantly different between SPMS and RRMS in the NAWM ($P = 0.008$). The mI in the NAWM was significantly different between SPMS and control groups, SPMS and RRMS respectively ($P = 0.003$ and $P = 0.001$). In the SPMS group, NAA(N-acetyleaspartate) and Cr were significantly different respectively ($P = 0.005$ and $P = 0.026$) between two areas of plaque and NAWM. In the subgroup of PPMS, the NAA showed a significant difference between the two areas ($P = 0.026$). The ratio of NAA/Cr in the white matter of the control group was significantly higher than the SPMS ($P = 0.002$) and PPMS ($P = 0.007$) plaques. The NAA ($P = 0.027$) and Cr($P = 0.007$) in patients with visual disorders was higher than patients with motor disorders.

Conclusion: The results of this study point to the fact that by checking the amount of Cr or NAA/Cr in MS plaques of RRMS and PPMS, patients can be diagnosed according to these MS subtypes. The two metabolites of NAA and Cr in brain plaques of patients with vision problems were higher than in patients with motor problems. There is a direct correlation between the increase of Cho in plaques and loss of balance in the patients. Increasing Cr and NAA in the NAWM have a direct relationship with visual disturbances as observed in patients and there is a direct correlation between the increase of NAA and sensory disorders in patients.

20 Sequencing Small-RNA Transcriptome of Individual Cells

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Small RNAs have been extensively studied and several small RNA classes have been identified. Current small RNA methods are limited to large number of cells. Here, we developed a novel method for sequencing small RNAs from individual cells and used it to profile naïve and primed human embryonic stem cells and cancer cells. Removing unwanted adaptor dimer ligations and blocking highly abundant rRNAs using masking oligo allowed us to reduce the input levels down to single cells and allowed us skip the common size-selection step, which makes this method automation friendly. Furthermore, by introducing a variant of unique molecular identifiers we were able to count small RNA molecules. We developed a computational pipeline to analyze data from potentially diverse classes of RNAs. As a result, the method captured mature and precursor RNAs of several classes including microRNAs, snoRNAs, tRNAs with their distinct length characteristics. In particular, single-cell micoRNA profiling stratified cell types robustly, indicating that single-cell small-RNA sequencing can be used to decode complex heterogeneous tissues. We envision that this method will open up for a wave of studies that will determine the small RNA landscape across rare cell types *in vivo*.

5
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