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LINP1 lncRNA expression profile is modulated in response to DNA damage

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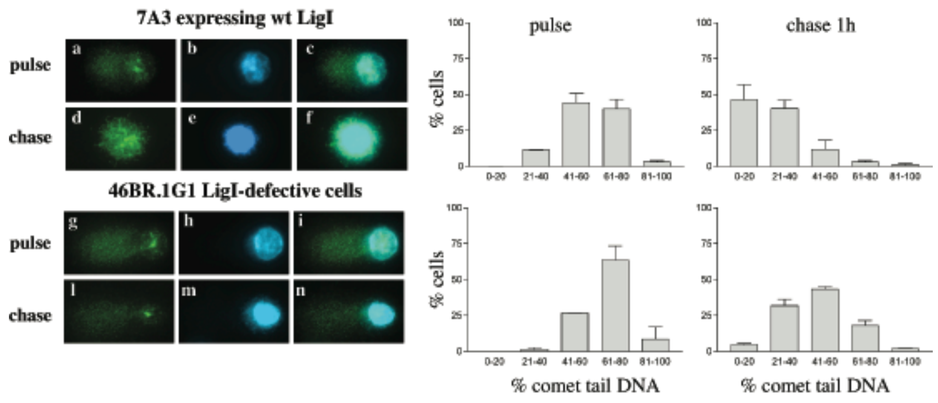
Background

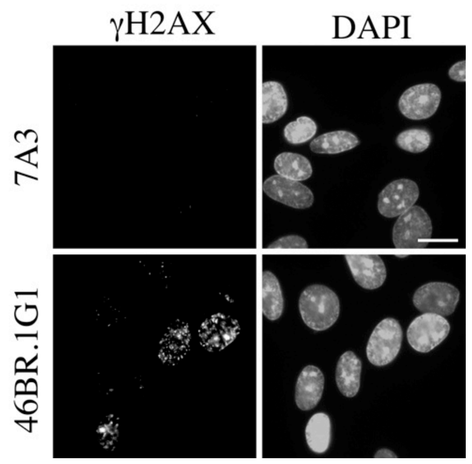
A low level of endogenous replicative DNA damage may impact gene expression programs and cell biology features relevant to cancer progression. This can be visualized by the comparison of DNA ligase I (LigI) defective 46BR.1G1 fibroblasts, deriving from a patient who died at 19 for lymphoma, and 7A3 cells, a 46BR.1G1 clone that stably expresses the ectopic wild-type LigI cDNA. LigI deficiency impairs maturation of newly synthesized DNA and increases the number of DSBs and γH2AX foci, two features associated with genome instability commonly found also in pre-neoplastic lesions.

46BR.1G1 cells do not complete the maturation of replicating DNA

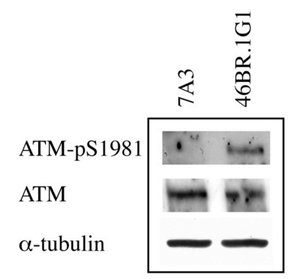


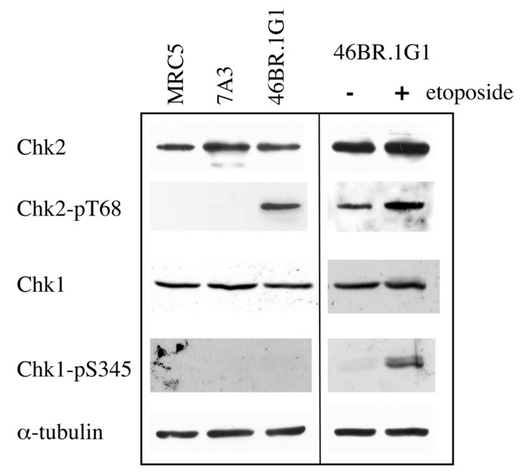
BrdU comet assay: cells were pulse- labeled with BrdU for 15 minutes and either immediately processed or chased for 1 hour. After 1 hour chase mature BrdU DNA was retained in the head of the comet (d) while in 46BR.1G1 LigI-defective cells newly synthetized DNA still migrates in the tail (I).

ATM/CHK2 pathway is constitutively activated in LigI defective cells



Representative images of γH2AX foci. Cells were analyzed in indirect immunofluorescence assay with anti-γH2AX ab and counterstained with DAPI.





Western blot analysis of total cell extract with antibodies against the indicated proteins or phosphoproteins. The same analysis was performed in 46BR.1G1 cells after etoposide treatment (et).

Despite the defect in DNA replication the ATR-Chk1 pathway is not activated and 46BR.1G1 cells proliferate in the presence of a low level of chronic DNA damage.

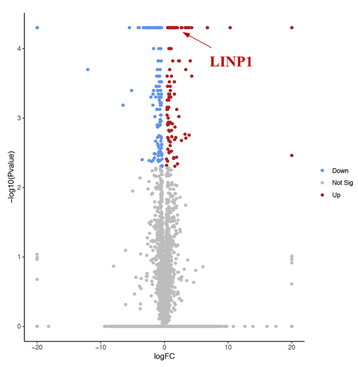
Aim

In order to decipher the strategy used to cope with replicative DNA damage, we have compared gene expression profiles in 46BR.1G1 and 7A3 cells. Among the differentially expressed genes, we identified a group of long noncoding RNAs (lncRNAs) which show significant transcriptional alteration in 46BR.1G1 cells, and appear to be relevant for cancer progression. We focused on *LINP1* (lncRNA in nonhomologous end joining (NHEJ) pathway 1) which is known to be involved in DNA repair.

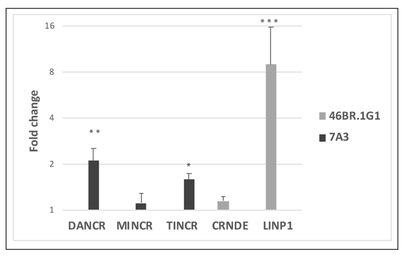
Results

LINP1 lncRNA is overexpressed in 46BR.1G1 cells

Aligned BAM files from RNA-seq were subjected to annotation according to the lncRNAs comprehensive annotation provided by GENCODE (n=13,870; release 19) and to gene quantification by the TopHat-Cufflinks protocol. A threshold of multiple testing corrected q-value < 0,05 was applied to define differentially expressed genes.

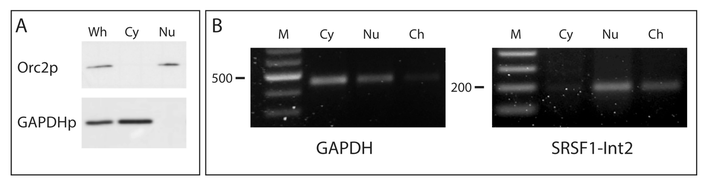


Volcano plot

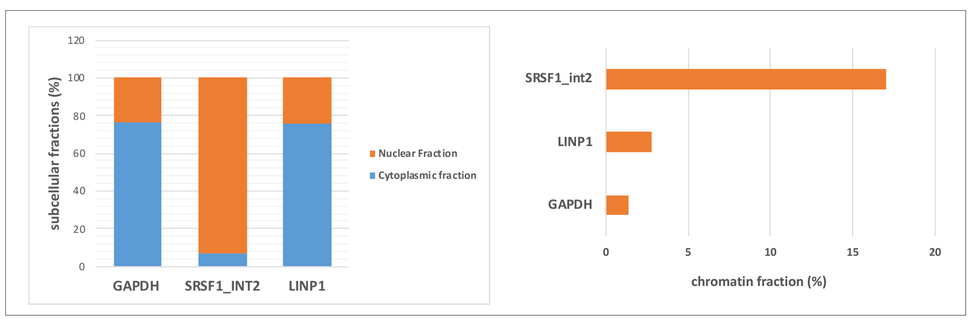


Real time RT-PCR: fold change was plotted on log_2 scale

LINP1 is mainly cytoplasmic however a fraction is chromatin bound in agreement with its role in DNA repair



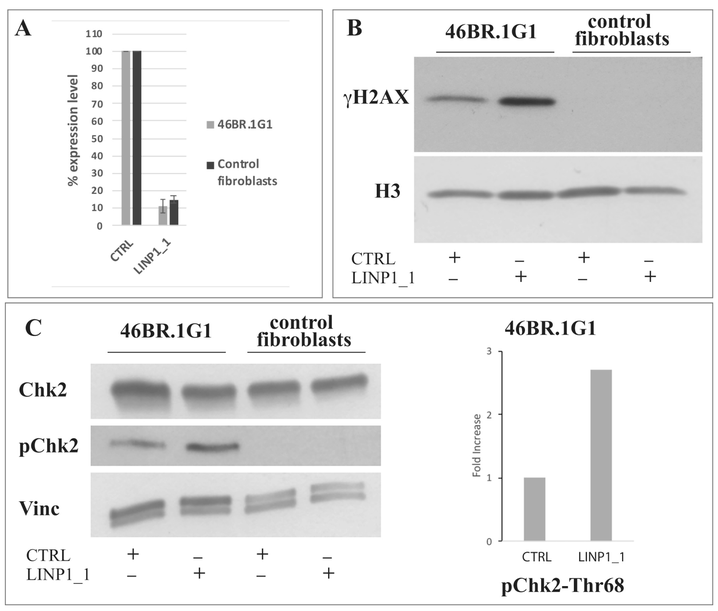
Control of 46BR.1G1 cells subcellular fractions



GAPDH transcript is mainly found in the cytoplasm while *SRSF1-Int2* is only detectable in nucleoplasmic and chromatin-rich fractions, as expected by the fact that introns are removed co-transcriptionally. *LINP1* is mostly in the cytoplasm, however, the fraction associated with chromatin, although drastically lower than *SFRS1-Int2*, is higher than that observed for *GAPDH*.

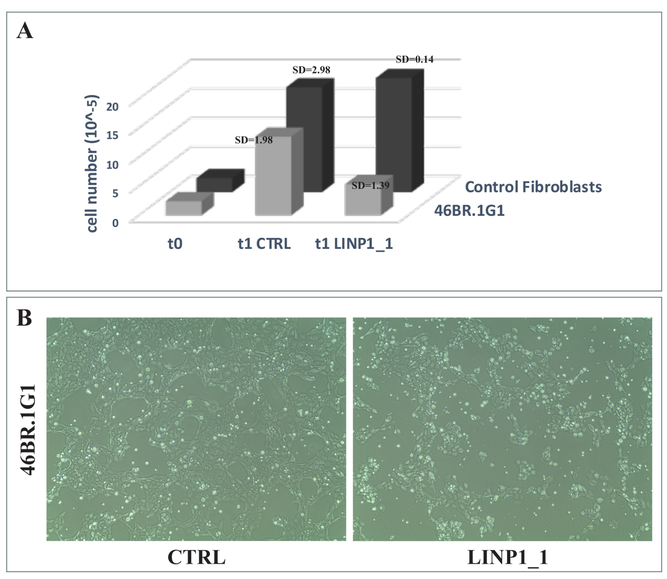
Subcellular and subnuclear distribution of *GAPDH*, *SRSF1-int2* and *LINP1* transcripts were analysed by real time RT-PCR.

LINP1 downregulation induces an increase of DDR markers in 46BR.1G1 cells



C: pChk2-Thr68 signal was normalized versus the amount of total Chk2 protein

LINP1 downregulation affects 46BR.1G1 proliferation



A: $2.4x10^5$ cells were transfected with control or LINP1_1 oligos and processed after 48 h. Data are the mean of 3 independent experiments. SD, standard deviation.
B: Representative phase contrast images of 46BR.1G1 cells 48 hours (t1) after transfection with CTRL and LINP1_1 LNA

These results indicate a functional role of *LINP1* expression in maintaining a sublethal level of DNA damage in 46BR.1G1 cells compatible with cell survival and proliferation.