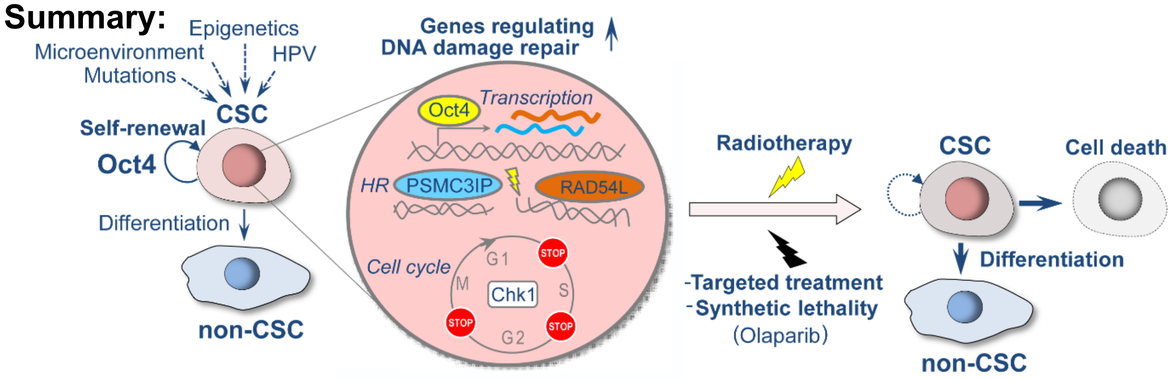
OncoRay – National Center for Radiation Research in Oncology, Medizinische Fakultät Carl Gustav Carus, TU Dresden, Fetscherstrasse 74, 01307 Dresden

The pluripotency transcription factor Oct4 contributes to head and neck squamous cell carcinoma radioresistance via regulation of DNA repair and the stem cell phenotype

Jacqueline Nathansen[1,2], Vasyl Lukiyanchuk[1,2], Linda Hein[1], Maya-Isabell Stolte[3], Kerstin Borgmann[3], Steffen Löck[1,4,5], Ina Kurth[1,6], Mechthild Krause[1,2,4,5,7], Michael Baumann[6] Annett Linge[1,4,5,7], Anna Dubrovska[1,2,4,7]

[1]OncoRay – National Center for Radiation Research in Oncology, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden, Germany; [2] Helmholtz-Zentrum Dresden-Rossendorf, Institute of Radiooncology-Oncoray, Dresden, Germany; [3]Laboratory of Radiobiology and Experimental Radiooncology, Center of Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [4]German Cancer Consortium (DKTK), partner site Dresden and German Cancer Research Center (DKFZ), Heidelberg; [5] Department of Radiotherapy and Radiation Oncology, Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany; [6] German Cancer Research Center (DKFZ), Heidelberg, Germany; [7] National Center for Tumor Diseases (NCT), partner site Dresden, German Cancer Research Center (DKFZ), Heidelberg; Faculty of Medicine and University Hospital Carl Gustav Carus, Technische Universität Dresden, and Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Dresden, Germany.

Summary:

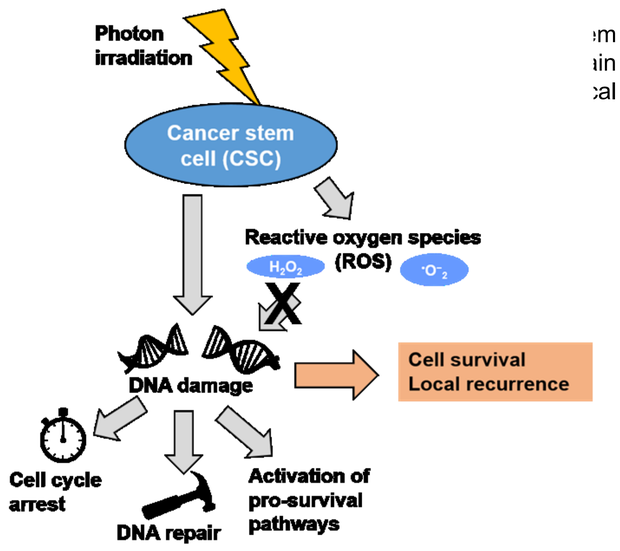


- Radiotherapy
- DNA

Figure 1: The pluripotency-related transcription factor Oct4 in HNSCC
- contributes to the CSC phenotype and radioresistance
- exerts its function by regulating the expression of important effectors of homologous recombination repair and cell cycle progression
- can be regarded as biomarker for radiotherapy-treated HNSCC patients and potential therapeutic target in combination with PARP inhibiton

Background: Despite being the sixth most common cancer type worldwide, head and neck squamous cell carcinoma (HNSCC) exhibits low five-year survival rates for advanced-stage patients.[1,2]

In contrast to HNSCC caused by human papillomavirus (HPV) infections, HPV-negative HNSCC cases often exhibit considerable resistance to radiotherapy.[3] Yet knowledge about radioresistance factors and potential therapeutic targets in HPV-negative HNSCC is limited. The local control probability after radiotherapy crucially depends on the eradication of cancer stem cells (CSCs), a sub-population of tumor cells characterized by pluripotency and an active DNA repair.[3,4]



This study provides evidence that the cancer stem cell (CSC)-related transcription factor Oct4 contributes to HNSCC radioresistance by regulating the DNA damage response and stem cell phenotype.

Understanding the role of Oct4 in HPV-negative HNSCC radioresistance is challenged by the existence of different Oct4 transcript variants and protein isoforms with presumably different functions.[6,7]

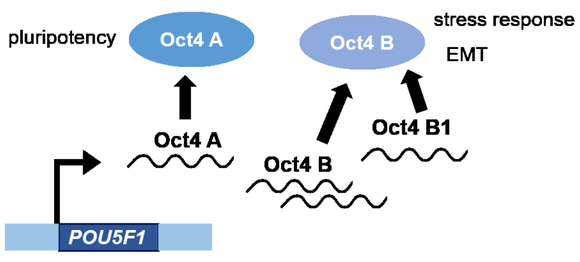


Figure 3: Diverging functions of the Oct4 isoforms A and B.

Cell models:

- siRNA-mediated knockdown of all Oct4 isoforms (total Oct4 knockdown) in Cal33 and UTSCC5 (HPV-negative HNSCC cell lines)
- CRISPR/Cas9-mediated Oct4 isoform A knockout in UTSCC5 cells

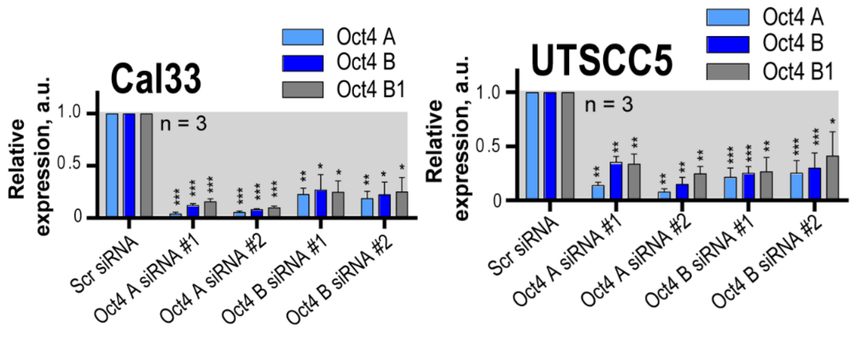


Figure 4: siRNA-mediated knockdown of Oct4 isoform A and B in HNSCC cell lines simultanously decreases expression of all isoforms.

Results:

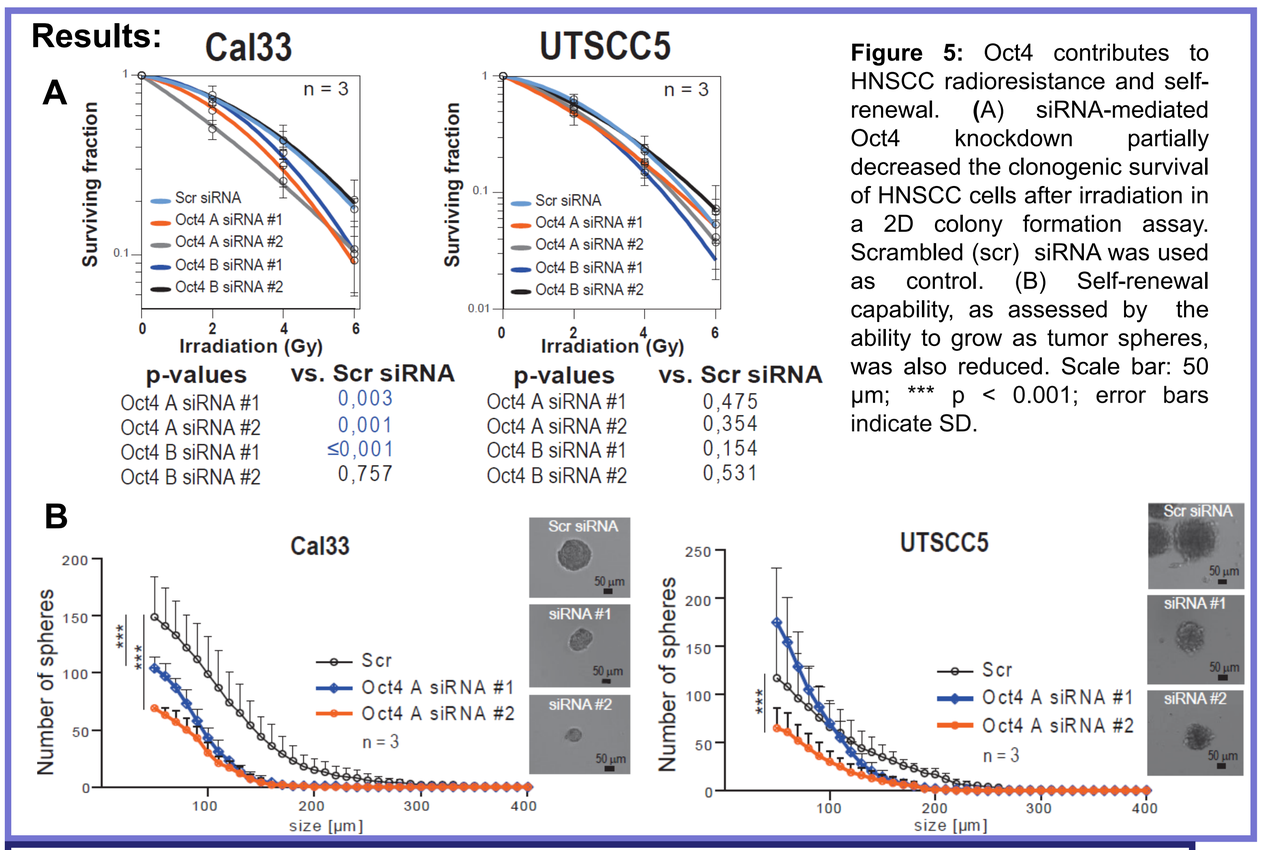
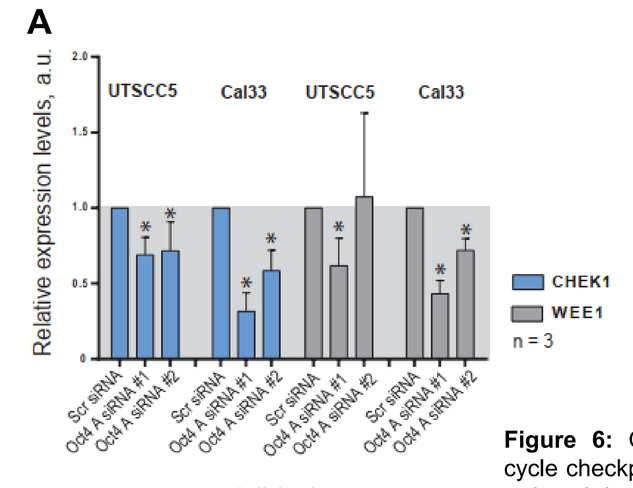
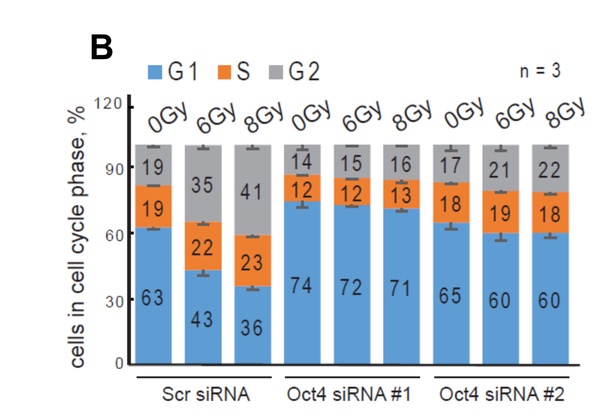


Figure 5: Oct4 contributes to HNSCC radioresistance and self-renewal. (A) siRNA-mediated Oct4 knockdown partially decreased the clonogenic survival of HNSCC cells after irradiation in a 2D colony formation assay. Scrambled (scr) siRNA was used as control. (B) Self-renewal capability, as assessed by the ability to grow as tumor spheres, was also reduced. Scale bar: 50 µm; *** p < 0.001; error bars indicate SD.





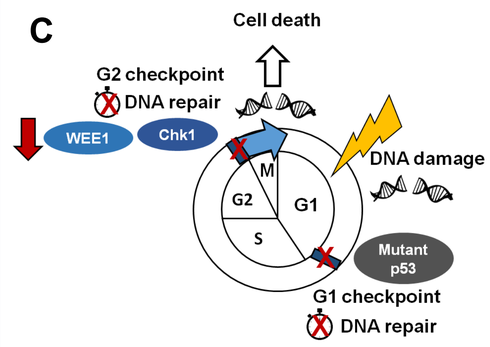


Figure 6: Oct4 downregulation impairs irradiation-induced cell cycle checkpoint activation. (A) siRNA-medited Oct4 A knockdown reduced the expression of the G2 checkpoint kinases CHEK1 and WEE1 in HNSCC cell lines. * p < 0.05; error bars indicate SD. (B) Consequently, UTSCC5 cells treated with siRNA against Oct4 fail to induce G2 cell cycle arrest after irradiation. An increase of G2 phase cells compared to 0 Gy was observed only in irradiated control (Scr siRNA) cells. # p < 0.05 compared to 0 Gy; error bars indicate SD. (C) Possible mechanism of radiosensitization by abrogation of the G2 checkpoint: HPV-negative HNSCC cells and tumors frequently harbor p53 mutations and lack a functional G1 checkpoint. Additional impairment of the G2 checkpoint could lead to cells entering mitosis with unrepaired irradiation-induced DNA damage and subsequently lead to cell death.[8,9]

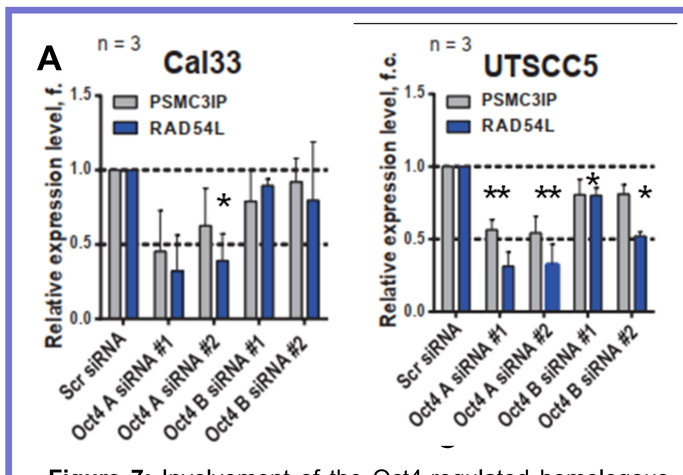


Figure 7: Involvement of the Oct4-regulated homologous recombination repair (HRR) genes PSMC3IP and RAD54L in HNSCC radioresistance and the CSC phenotype. (A) PSMC3IP and RAD54L expression levels are reduced in response to Oct4 knockdown. siRNA-mediated knockdown of PSMC3IP and RAD43L significantly reduced 2D clonogenic cell survival after iradiation (B) as well as self-renewal capability assessed by sphere formation assay (C). * p < 0.05; error bars indicate SD.

Figure 8: PARP inhibition selectively radiosensitizes Oct4 A depleted UTSCC5 cells. Pre-treatment with the PARP inhibitor Olaparib 2h before irradiation significantly reduced 2D clonogenic survival after irradiation only in UTSCC5 cells with CRISPR/Cas9-mediated Oct4 A knockout (KO1). Wild type (WT) cells exhibit no significant radiosensitization by PARP inhibition, indicating a HRR deficiency only in Oct4 A-depleted cells. * p < 0.05; error bars indicate SD.

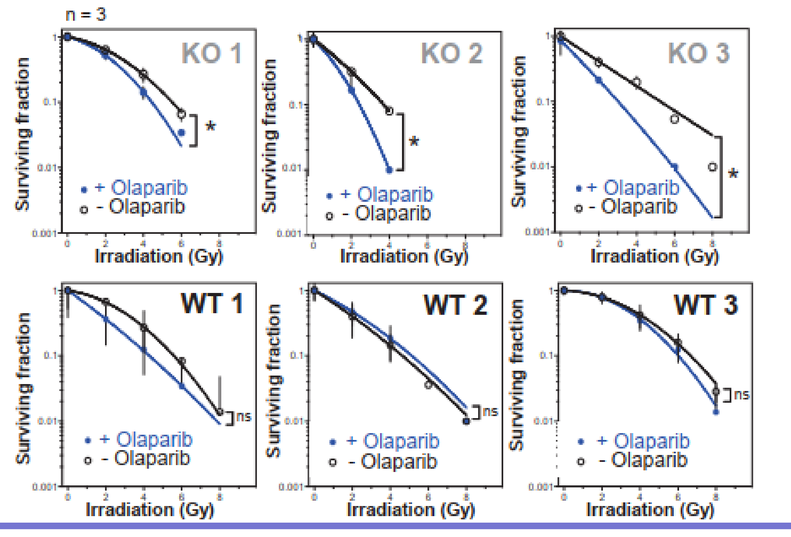
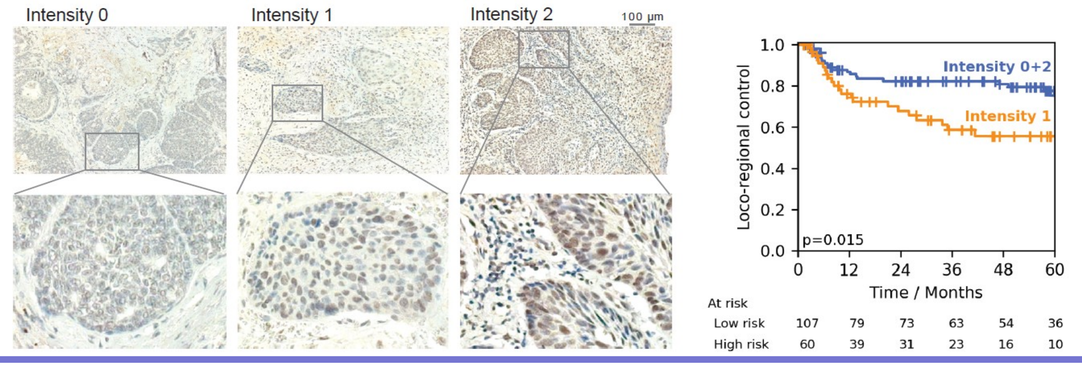


Figure 9: Kaplan-Meier analysis of patients treated with postoperative radio(chemo)therapy (PORT-C). High and low nuclear Oct4 expression at the invasive front is associated with poor loco-regional control; n = 167.



Conclusion: Our results in HPV-negative HNSCC cell models emphasize the interplay between DNA repair factors and the HNSCC CSC phenotype. The involvement of Oct4 in the regulation of DNA repair and cell cycle progression provides new insights into HNSCC radioresistance and opens possibilities for combination therapy with PARP inhibitors.

References

1.Leemans et al., Nat. Rev. Cancer 2011; 2.Pulte and Brenner, Oncologist 2010; 3.Linge et. al, Clin. Cancer Res. 2016; 4.Baskar et al. Front Mol Biosci. 2014; 5.Krause et al., Adv Drug Deliv Rev. 2017; 6.Wang and Dai, Stem Cells 2010; 7.Lin et al., Oncogene; 8.Hein et al., Int J Oncol. 2014; 9.Zhang and Hunter, Int J Cancer 2014

Acknowledgement: The authors thank Liane Stolz-Kieslich and the team of The Core Facilities of the Technology Platform at BIOTEC TU Dresden for technical support. The work was partially supported by DFG, Wilhelm-Sander-Stiftung, BMBF, Hubertus Wald Stiftung, and Hamburger Krebsgesellschaft e.V.

Contact: jacqueline.nathansen@tu-dresden.de; anna.dubrovska@oncoray.de