SPOT-003 THYMIDYLATE SYNTHASE MAINTAINS THE UNDIFFERENTIATED STATE OF AGGRESSIVE BREAST CANCERS

[1]P Ceppi*, [1]A Siddiqui, [1,2]P Gollavilli, [3]D Pluim, [4]O Saatci, [5]L Annaratone, [2]I Asangani, [3]JH Schellens, [5]C Marchio, [4]O Sahin. [1]*University Clinic Erlangen, Interdisciplinary Center for Clinical Research, Erlangen, Germany*; [2]*University of Pennsylvania, Department of Cancer Biology- Perelman School of Medicine, Philadelphia, USA*; [3]*Netherlands Cancer Institute, Pharmacology, Amsterdam, Netherlands Antilles*; [4]*Bilkent University, Department of Molecular Biology and Genetics, Ankara, Turkey*; [5]*University of Turin, Pathology Unit-Department of Medical Sciences, Turin, Italy*

10.1136/esmoopen-2018-EACR25.37

Introduction De-differentiation is a highly lethal feature of aggressive breast cancers (BC), and is achieved through the epithelial-to-mesenchymal transition (EMT) and the cancer stem cell (CSC) programs. Targeting the mechanisms controlling BC de-differentiation can lead to more effective therapeutics. Recent studies indicated that nucleotide metabolism can regulate cancer stemness and EMT. Here we investigated the expression of the nucleotide metabolism enzyme and drug target thymidylate synthase (TS) in the BC subtypes and analysed its impact on BC de-differentiation.

Material and methods Cells with TS knockdown and overexpression were tested *in vitro* and *in vivo*. Proteins were analysed by western blot, FACS and ELISA. Differential gene expression in TS-deficient cells was determined by RNA-seq. Immunohistochemistry (IHC) was used to stain samples from patients with different BC subtypes.

Results and discussions TS mRNA expression was found to be significantly differentially expressed among the BC subtypes, exhibiting the highest levels in aggressive triple-negative BC (TNBC). shRNA-mediated TS knockdown in TNBC cell lines (n=3) increased the population of differentiated cells ($CD24^{high}$) and strongly attenuated the stem-like phenotype, like the formation of mammospheres from single cells and the migration in a cell culture wound. TS-deficient cells also showed an altered ability to form metastasis *in vivo*, consistent with previous observations in EMT-repressed BC cells. A rescue experiment performed by overexpressing either a wild-type or catalytically inactive TS indicated that the enzymatic activity was essential for the maintenance of the BCSC phenotype. Along with a strong repression of EMT-signature genes, RNA-seq profiling indicated a reduction of inflammatory and NF-κB signalling pathways in TS deficient cells, which dramatically reduced IL-1β production and secretion. A TS-specific gene signature was generated, which significantly associated with worst survival in BC patients. IHC staining on FFPE samples from a series of BC patients (n=120) confirmed higher TS expression in tumours that were poorly differentiated and in TNBC.

Conclusion We discovered a novel role for the TS enzyme in the maintenance of a de-differentiated and stem-like state of BC. These findings may not only open the possibility to study in-depth the role of nucleotide metabolism at the crossroad between proliferation and differentiation, but may provide the *rationale* for novel drug combinations with TS-inhibiting agents for the treatment of BC.

SPOT-004 NEDD9 IS CRUCIAL FOR TUMOUR PROGRESSION IN RENAL CELL CARCINOMA MOUSE MODELS

M Henjakovic, M Cherviakova, J Schleifenbaum, A Euteneuer, N Mikhael, A Nikiforov, L Thelen, T Seeger-Nukpezah*. *University Hospital of Cologne, Department I of Internal Medicine, Cologne, Germany*

10.1136/esmoopen-2018-EACR25.38

Introduction Renal cell carcinoma (RCC) is characterised by high lethality in advanced stages. Vastly resistant to radio- and chemotherapy, RCCs respond to targeted therapies such as tyrosine kinase inhibitors, mTOR antagonists and immune checkpoint inhibitors. However, limited response rates and emerging resistance mechanisms demand new treatment strategies.

Scaffolding protein NEDD9 (neural precursor cell expressed, developmentally down regulated 9) is frequently overexpressed in various cancer types and associated with tumour aggressiveness. *In vivo*,high NEDD9 expression is associated with adverse clinical outcome and *in vitro*, NEDD9 promotes aggressiveness in RCC cells. Thus, we systematically characterised the role of NEDD9 in RCC both *in vitro* and *in vivo* with the goal to establish basis for new treatment strategies.

Material and methods Using RNAi, we generated NEDD9-proficient and -deficient syngeneic (RENCA) and xenograft (786-O) tumour models via subcutaneous and orthotopic transplantations as well as tail vein injections. Tumour growth was monitored dynamically using calliper and MRI imaging. Extensive *in vitro* studies were performed to analyse NEDD9 and its associated oncogenic signalling cascades including Western blot, proliferation, migration and gene expression analyses. Tissue microarrays (TMAs) of 92 RCC patients were stained for NEDD9 and analysed using automated quantitative analysis (AQUA) technology to associate NEDD9 protein expression with clinical outcome.

Results and discussions NEDD9 is highly expressed in RCC cells and NEDD9 depletion significantly impairs migration and proliferation in both human and murine RCC cells. This is accompanied by reduced signalling of oncogenic pathways, particularly activation of ERK1/2. NEDD9 tumour promoting role was confirmed *in vivo* where NEDD9-deficient murine RCC cells exhibited significantly reduced tumour growth after subcutaneous and orthotopic syngeneic transplantation as well as inhibited lung metastatic seeding capacity after tail vein injection.

In human RCC cells, only NEDD9 down-regulation was sufficient to completely abolish tumour growth in subcutaneous, orthotopic and lung seeding xenograft models. In line with our *in vitro and in vivo* results, we found high NEDD9 expression in human RCC tissue to be significantly associated with shorter overall survival.

Conclusion We show for the first time a crucial role for NEDD9 in RCC tumour progression *in vivo*, which potentially offers new therapeutic approaches.