typical features of high-grade serous ovarian cancer. We believe that OVPA8 cell line is a good model to be used in the studies of this type of cancer.

PO-135 ANALYSIS OF THE TRANSFORMING ACTIVITY OF HUMAN CANCER IDENTIFIED VAV1 MUTANTS PROVE ITS CLASSIFICATION AS AN ONCOGENE

[1]M Farago*, [2]B Shalom, [1]E Pikarsky, [1]S Katzav. [1]*Hebrew University, Institute for Medical Research Israel-Canada- Hadassah Medical School, Jerusalem, Israel*; [2]*Hebrew University, Institute for Medical Research Israel-Canada- Hadassah Medical School, Jerusalem, Israel*

10.1136/esmoopen-2018-EACR25.176

Introduction The activity of Vav1 as a mutant oncogene in human tumours has remained questionable for decades. Although mutants of Vav1 were recently identified in human cancers of various origins, the functional activity of these mutants is not fully studied. Vav1 is physiologically active as a GDP/GTP nucleotide exchange factor (GEF) in the hematopoietic system. In this study, we addressed the contribution of several cancer-identified Vav1 mutants to tumorigenic processes.
Material and methods We introduced several amino-acid substitutions at residues identified in human lung cancer as follows: glutamic acid (position 59; calponin homology region) to lysine (E59K); aspartic acid (position 517; C1 domain) to glutamic acid (D517E); and leucine (position 801; carboxySH3) to proline (L801P). The biochemical and transforming activities of these mutants were tested following transfection into NIH3T3 cells.
Results and discussions Among the mutants produced, E59K generated a truncated protein, which preserved its expected size once cells are incubated with MG132, a specific proteasome inhibitor. E59K, D517E and oncVav1 are active as GEF towards Rho/RacGTPases, albeit E59K exhibited the uppermost activity. This result was illustrated by Pymol, a computer software for molecular visualisation, that predicts its increased activity as a GEF. This activity is also manifested in changes in cytoskeleton organisation indicative of transformation. Analysis of protein stability using cycloheximide decay assay revealed that D517E mutant protein is more stable than the other mutants, thus explaining its increased expression and activity. Furthermore, NIH3T3 cells expressing E59K, D517E and oncVav1 mutants exhibit increased cell proliferation, elevated number of transformed foci and increased number of generated tumours in NOD/Scid mice. Of note is the fact that tumours generated by E59K exhibit the most aggressive phenotype among the mutant proteins used in this study, reminiscent of epithelial morphology.
Conclusion Our results convincingly attest to the transforming potential of the Vav1 mutants, E59K and D517E, thus providing compelling evidence that Vav1 mutants can act as 'real' oncogenes in human cancer.

PO-136 SOX2 IS A NOVEL BIOMARKER FOR HIGH-RISK EWING SARCOMA

[1]G Sannino*, [1]M Aruna, [1]C Zacherl, [1]R Alba Rubio, [2]MM Kiran, [3]G Hardiman, [4]T Kirchner, [1]M Orth, [1]S Ohmura, [1]TGP Grünewald. [1]*Institute of Pathology- Faculty of Medicine- LMU Munich, Max-Eder Research Group for Pediatric Sarcoma Biology, Munich, Germany*; [2]*Inonu University, Department of Pathology- Turgut Ozal Medical Center, Malatya, Turkey*; [3]*Medical University of South Carolina, Departments of Medicine- Public Health and Center for Genomics Medicine, Charleston- South Carolina, USA*; [4]*Institute of Pathology, Faculty of Medicine- LMU Munich, Munich, Germany*

10.1136/esmoopen-2018-EACR25.177

Introduction Ewing sarcoma (EwS) is the second most common paediatric bone-associated cancer, which may originate from mesenchymal stem cells (MSCs). It is characterised by specific fusion oncogenes involving the *EWSR1* gene and variable members of the *ETS* family of transcription factors (mostly *FLI1*). EWSR1-FLI1 is an aberrant transcription factor, which deregulates hundreds of genes via creating *de novo* enhancers at GGAA-microsatellites. Previous data showed that ectopic *EWSR1-FLI1* expression in MSCs induces *SOX2*. SOX2 is a stem cell marker, whose precise function in EwS remains elusive.
Material and methods We employed DNA microarrays, RNA-Seq, ChIP-Seq, qRT-PCR, Western blot, immunohistochemistry, RNA-interference, *in vitro* and *in vivo* functional assays.
Results and discussions Analysis of gene expression and tissue microarrays revealed that SOX2 is highly expressed only in 15%–20% of EwS tumours. In two cohorts, SOX2-high patients had very poor outcome, which appeared to be independent from typical clinical markers for worse outcome such as age and metastasis. The observed heterogeneity of SOX2 expression was not attributable to differential methylation levels and copy number changes at the *SOX2* promoter. However, in cell culture models we found that knockdown of *EWSR1-FLI1* in EwS cells reduces *SOX2* expression, whereas its ectopic expression in embryonic stem cells up-regulated *SOX2*. We currently investigate the potential inter-patient variability of EWSR1-FLI1 bound *SOX2*-regulatory elements such as GGAA-microsatellites, which may explain differential SOX2 expression across EwS tumours. To elucidate the role of SOX2 in EwS, we performed gene-set enrichment analysis of *SOX2* co-expressed genes in 117 primary EwS tumours, which indicated that SOX2 may be involved in proliferation, stemness and drug resistance. In agreement, knockdown of *SOX2* in EwS cell lines markedly reduced cell proliferation and clonogenic growth *in vitro* as well as tumour growth *in vivo*. Using combined RNA-Seq and ChIP-Seq experiments, we identified potential SOX2 targets, which may collectively explain the SOX2 phenotype. We are currently screening a drug library to identify a novel treatment options for SOX2-high EwS patients.
Conclusion Taken together, our data suggest that *SOX2* is a heterogeneously expressed EWSR1-FLI1 target gene, whose high expression confers an aggressive phenotype, and thus may serve as a biomarker for a high-risk subgroup of EwS patients.

PO-137 LIVER SPECIFIC NEMO ABLATION INDUCES MIXED HEPATOCELLULAR-CHOLANGIOCARCINOMA IN MYC-OVEREXPRESSING MICE

[1]J He*, [1]M Gerstenlauer, [2]F Leithaeuser, [3]MM Yeh, [1]LK Chan, [1]HJ Maier, [1]T Wirth. [1]*Physiological Chemistry, Medical department, Ulm, Germany*; [2]*Pathology Institute, Pathology Department, Ulm, Germany*; [3]*Anatomic and Clinical Pathology, Pathology Department, Seattle, USA*

10.1136/esmoopen-2018-EACR25.178

Introduction The MYC oncogene appears to be critically involved in the pathogenesis of HCC. The role of NF- κB in liver cancer is complex, either acts as a tumor-promoter or tumor-suppressor in liver carcinogenesis. However, the role of NF- κB in MYC-induced liver carcinogenesis has not been reported. In the present study, we have generated a mouse model of MYC-induced HCC with or without liver specific