Molecular Oncology

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# EACR 2023 Congress Abstracts

## Contents



### Proffered Papers

10-minute talks awarded for the highest scored abstracts, embedded in the scientific symposia sessions. These presentations are not accompanied by a poster.

### Posters in the Spotlight

Tuesday 13 June, 17:30 - 18:30, Poster and Exhibition Hall
Wednesday 14 June, 17:15 - 18:15, Poster and Exhibition Hall

Dedicated sessions taking place in the spotlight area within the Poster and Exhibition Hall. Poster presenters with high scoring abstracts will give short presentations of up to 10 minutes. Their posters will also be available to view during the Poster Discussion Sessions.

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pLKO-shYAP. Metabolic and non-metabolic viability assays were used to assess the sensitivity to the $NAD^+$-depleting agent FK866 in attachment conditions, while the soft agar assay was performed to evaluate the ability of cells to grow in anchorage-independent conditions. Mitochondrial respiration was assessed through the Seahorse MitoStress test. Real-Time PCR and Western Blot were performed to determine the expression levels of genes/proteins of interest.

**Results and Discussions**

We showed that FK866 treatment decreases YAP phosphorylation status at serine 127, thus increasing its nuclear translocation in a cancer cell model of TNBC, which is sensitive to FK866. The stable silencing of YAP in MDA rescued FK866 toxicity both in 2D and 3D culturing, promoting a dose-dependent FK866-resistant like phenotype. At the metabolic level, the silencing of YAP on MDA p. induces the FK866-dependent expression of the mitochondrial biogenesis gene PGC1-α and increases the mitochondrial respiratory capacity. These features were previously associated with TNBC FK866-resistant phenotype by our group.

**Conclusion**

We identified a correlation between YAP activation and sensitivity to FK866, which can partially sustain the acquirement of resistance to FK866 in TNBC, likely through the modulation of mitochondrial metabolic traits.

## EACR23-1161
## Genetically and phenotypically heterogeneous cancer stem cells can be isolated from single glioblastomas by alternative selective pressures

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**Introduction**

Glioblastoma (GBM) is an invariably lethal brain tumor, known for its genetic and transcriptional heterogeneity, which limit the effectiveness of current treatments. GBM contains a population of stem-like cells (GSCs), essential for tumor onset, progression, and therapeutic resistance. GSCs are typically isolated from patient biopsies using a culture technique producing "neurospheres" (NS) in a serum-free medium supplemented with EGF and FGF2. The current methodology mostly relies on tissue samples of limited size, which may not fully capture the entire range of GBM heterogeneity. Moreover, standard culture conditions may select for a limited number of subclones that have the highest fitness in those conditions, potentially leading to an incomplete picture of the genetic diversity of the tumor. Hence, there is a need for alternative methods of GSC isolation that can better capture GBM heterogeneity and facilitate the study of subclonal evolution within the tumor.

**Material and Methods**

We collected a cohort of human GBMs surgically removed as ultrasonic aspirates (UA; n=31), virtually allowing to recover the entire tumor mass. From each single UA we established multiple NS parallel cultures ('NS families') by applying different positive selective pressures, represented by different cocktails of growth factors (EFG, FGF2, PDGFBB, and HGF). Four representative NS families underwent full genomic, transcriptomic and phenotypic characterization.

**Results and Discussions**

Within each NS family, NS members displayed homogeneous driver gene mutations but, when present, heterogeneous EGFR or MYC gene amplifications, in terms of amplification levels and modalities (clusters vs. double minutes). However, the tumorigenic potential of each NS wasfound to better correlate with its transcriptional profile rather than its genetic landscape. Across multiple GBMs, classical NS were invariably more malignant than mesenchymal GSCs *in vitro* and *in vivo*. Interestingly, from a tumor including two histopathologically distinct areas, a conventional GBM and a more aggressive primitive neuronal component, we established a NS family whose members displayed homogeneous genetic features but distinct transcriptional and biological features, and were able to reproduce one or the other tumor's component.

**Conclusion**

Our methodology allows to propagate distinct GSCs from individual tumors, which, collectively, better recapitulate the original GBM and represent a resource to study GBM heterogeneity.

## EACR23-1162
## Expression profiling of ANKRD1 in rhabdomyosarcoma cell lines

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**Introduction**

Rhabdomyosarcoma (RMS) is the most common soft tissue malignancy in children and adolescents. Respecting the age of the patients and the tumor aggressiveness, investigation of the molecular mechanisms of RMS tumorigenesis is essential, most notably due to the possible identification of novel therapeutic targets. To contribute to a better understanding of the molecular pathology of RMS, we investigated ANKRD1 (ankyrin repeat domain 1) gene, considered a potential RMS diagnostic marker. The changes in its expression are related to carcinogenesis and resistance to chemotherapy in several types of tumors.

**Material and Methods**

In this study, we used three RMS cell lines, SJRH30, RD and HS-729. The expression pattern of endogenous ANKRD1 was determined by RT-PCR, qPCR, Western blot and immunoprecipitation, and the intracellular localization of the protein was examined by immunocytochemistry. Sanger sequencing was used to check for possible alterations in the ANKRD1 open reading frame. Transient transfections of cells were performed with the eukaryotic expression vector encoding ANKRD1 open reading frame. Proteasomal degradation was inhibited by the incubation of cells with MG132.

**Results and Discussions**

RMS cell lines expressed similar amounts of wild-type ANKRD1 transcript, but the protein level was different. ANKRD1 protein was expressed at detectable levels in the SJRH30 and RD cells (SJRH30>RD), but not in the HS-729. Immunocytochemistry revealed the predominant nuclear localization of ANKRD1 and its presence in unidentified nuclear bodies. Overexpression of ANKRD1 was not achieved in RMS cell lines, although it was successful in other cell types. This was potentially due to the proteasomal degradation of ANKRD1, as inhibition of proteasomes with MG132 led to an increase of ANKRD1 protein level in the RMS cells.

**Conclusion**

ANKRD1 protein is expressed at different levels in RMS cell lines and its regulatory role is supported by nuclear localization. ANKRD1 propensity for proteasomal degradation indicates that ANKRD1 overexpression may be lethal for RMS cells. These observations suggest that ANKRD1 warrants further consideration as RMS therapeutic target.

## EACR23-1165
## Deregulation of BID in malignant thyroid neoplasm

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**Introduction**

BID (BH3 Interacting Domain Death Agonist) is a proapoptotic protein belonging to the BCL-2 family that plays a central role in apoptotic signaling pathway. Several papers reported that deregulated expression of BID is associated with poor outcome in cancer patients. Although the majority of papillary thyroid cancer (PTC) patients respond well to therapy, no effective therapeutic strategies are available for the treatment of the aggressive forms of this neoplasm. Given the importance of the apoptotic process during the human carcinogenesis, we purpose to assess the expression of BID in thyroid cancer (TC) tissues.
microRNAs (miRNAs), regulating gene expression, have an important role in the molecular pathogenesis of TC. In this field, our preliminary data showed that BID is one of downregulated proteins in TC cells overexpressing the miR-331-5p, suggesting that this miRNA could regulate the expression of BID in this malignancy.

**Material and Methods**

Expression studies in thyroid cancer tissues were investigated by *in silico* analysis. Bioinformatic program, western blot and luciferase assays were applied to unveil that BID is a direct target of miR-331-5p.

**Results and Discussions**

By interrogating The Cancer Genome Atlas (TCGA-THCA), we found that BID is overexpressed in thyroid cancer compared to normal thyroid tissues. The ectopic expression of miR-331-5p in thyroid cancer cells leads to the downregulation of BID protein. The luciferase assay confirmed the direct targeting. Co-expression analysis across 509 thyroid cancer tissues unveils an inverse correlation between miR-331-5p and BID. Interestingly, the proliferation marker KI67 is inversely correlated with miR-331-5p and positively correlated with the mRNA of BID.

**Conclusion**

These altogether findings suggest that the altered expression of BID/miR-331-5p could be a novel potential candidate biomarker for the malignant thyroid neoplasm.

## EACR23-1180
## Engineering Target Tissue in Lab-on-a-chip Devices for Predicting Homing Choices of Metastatic Cancer

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**Introduction**

Breast cancer is the most common cancer in women, and lung is one of the primary sites of metastasis. Metastasis is the leading cause of cancer related deaths. One of the most important steps of the metastatic cascade is the extravasation of cancer cells into distant organs. Vascularization is a critical component of engineering the tumor microenvironment. Recently developed lab-on-a-chip platforms strive to mimic the in vivo