loss of mesenchymal markers and acquisition of epithelial markers. CNN3 partially restored the colon cancer cell sensitivity to 5FU. CNN3 was expressed in 20/56 (39%) of FFPE colon cancer by immunohistochemistry and it was not related to p53 stabilisation consistent with our cell line findings.

Conclusion The results suggested involvement of CNN3 in EMT and hence metastasis and also in resistance to standard chemotherapy in colon cancer. These data deserves further exploration in vivo and in clinical studies to validate the potential clinical applications of CNN3 in cancer treatment and prognosis.

PO-207 THE ROLE OF RSU-1 IN GLIOMA CELLS METASTASIS

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Introduction Ras Suppressor-1 (RSU-1) was recently found to be associated with Focal Adhesion (FA) proteins. The main objective of this research work was the *in vitro* characterisation of a panel of glioma cancer cells in terms of aggressiveness, and the investigation of RSU-1 role on invasion of glioma cancer cells.

Material and methods A panel of four human neuroblastoma cell lines was used (H4, SW1088, A172, U87-MG). Invasion assay with matrigel-coated transwell and soft agar growth assay were performed in order to characterise the aggressiveness of glioma cell lines. The RSU-1 expression for glioma cells was tested by real time PCR and immunoblotting. Finally, glioma cells were transfected with siRNA against RSU-1 in order to find out the role of RSU-1 in glioma cells.

Results and discussions In order to assess the invasive potential of the four glioma cell lines, a transwell invasion assay was performed and A172 and U87-MG cells found to be more invasive than H4 and SW1088 cells. To determine the aggressiveness of the studied cell lines, soft agar assay was also performed. The number of colonies for the U87-MG and A172 was significantly larger than the H4 and SW1088, with the latter cell lines only forming a few small colonies.

We then sought to find out whether RSU-1 gene is differentially expressed in the four cell lines and whether its expression is correlated with invasiveness. It was found that the more aggressive A172 and U87-MG cell lines, overexpress RSU-1 compared to the less aggressive H4 and SW1088 cell lines.

Subsequently, two cell lines were selected to be used for further experiments, H4 and A172 which are the least aggressive and more aggressive cells, respectively. Our results show that upon RSU-1 silencing, the invasion of A172 cells was significantly decreased whereas invasion of H4 cells was increased with respective changes observed in the expression of Matrix Metalloproteinase 13 (MMP13), a fundamental protease in cancer cell metastasis.

Conclusion Results confirmed that the A172 and U87-MG glioma cells are more aggressive than H4 and SW1088. Also, RSU-1 was found to be overexpressed in most aggressive cells in comparison to less aggressive cell lines. More aggressive A172 cells lacking RSU-1 showed decreased invasion while H4 cells showed increased invasion. Collectively, RSU-1 found to be critical for glioma cell invasion and further investigation of the implicated molecular mechanism is underway.

Ageing and Cancer

PO-208 EMERGING ROLES OF HECT TYPE E3 UBIQUITIN LIGASE SMURF2 IN CANCER

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Introduction Despite significant advances made in the treatment of specific cancer types, eradicating the disease, especially in its most dangerous metastatic forms, has yet to be achieved. The lack of an effective treatment together with the high mortality rates of the patients emphasise the urgency to explore novel therapeutic targets and paradigms, which can subsequently be incorporated in cancer treatment.

Recently, we discovered Smurf2 – an E3 ubiquitin ligase, chromatin modifier and signal transduction regulator, as a key cellular factor operating in cells to prevent cell transformation and carcinogenesis. We have also reported that Smurf2 acts as an important regulator of DNA damage response (DDR), gene expression, and genomic integrity maintenance spanning through and interlocking these components. Blank M et al., *Nature Med* 2012. These findings prompted us to further investigate and characterise molecular processes operating under Smurf2 control in mammalian cells.

Material and methods A gamut of different approaches ranging from genetics and biochemistry (including mass spectrometry analysis) through molecular cell biology to animal pathophysiology and human sample analyses (conducted on a variety of human normal and cancer cells and tissues) were used in our study.

Results and discussions Our research efforts identified a novel mechanism by which Smurf2 regulates genome integrity maintenance – through stability regulation of DNA topoisomerase IIα (Topo IIα) – one of the key cellular enzymes in chromatin organisation, dynamics and unaltered chromosome inheritance. We discovered that Smurf2 stabilises Topo IIα and prevents the formation of pathological DNA bridges; as well as modulates cell sensitivity to Topo IIα-targeting drug etoposide (Emanuelli et al. *Cancer Res* 2017).

Our second line of investigation led to elucidation of Smurf2 as a *bona fide* negative regulator of nuclear A-type lamins, in particular of lamin A and its disease-associated form progerin (Borroni et al. *Ageing Cell* 2018). Both lamin A and progerin have been connected to genome integrity maintenance, DDR and gene expression, and linked to cancer. This association suggests that the targeting of A-lamins in cancer might be a promising direction to eradicate tumour cells, but more fundamental work is needed.

Conclusion Altogether, our findings support the notion that Smurf2 is intrinsically involved in the regulation of pivotal molecular and cellular processes operating in mammalian cells and leading to cancer.

PO-209 BIOLOGICAL CHARACTERISATION OF SPECIFIC KRAS MUTATIONS: FROM BASIC BIOLOGY TO RESPONSE TO TREATMENT

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Introduction KRAS is one of the most frequently mutated oncogenes in cancer and KRAS mutations are commonly associated
with resistance to therapy and poor prognosis. KRAS is still not directly druggable, therefore current therapeutic strategies for KRAS mutant cancers aim at identifying susceptibilities in downstream signalling pathways. One unresolved aspect of KRAS biology with potential to translate into patient stratification criteria is the difference between distinct KRAS activating mutations in terms of downstream signalling and drug sensitivity. Understanding the biochemical and biological differences among specific KRAS mutants is essential to discover new actionable vulnerabilities for mutant KRAS.

**Material and methods** To study the role of different KRAS mutants in a controlled and reliable genetic setting, we developed an isogenic KRAS\(^{\text{MUT}}\) inducible system that lacks endogenous HRas/NRas and harbours conditional CRE\(^{\text{ERT2}}\)-controlled KRas\(^{\text{lox}}\) alleles (KRas\(^{\text{lox}}\) KRAS\(^{\text{MUT}}\) system). This system allows direct and robust comparison between different KRAS oncogenic isoforms and rigorous evaluation of the in vitro and in vivo impact on tumour progression and response to MEK/ERK inhibition.

**Results and discussions** Our data confirm differential GTP-hydrolysis properties among different KRAS mutants (G12C, G12D, G12V, G12A, G13D and Q61H). This intrinsic feature is translated into distinct proliferation rates in vitro and in vivo, as well as differential responsiveness to MEK/ERK inhibitors in specific KRas\(^{\text{lox}}\) KRAS\(^{\text{MUT}}\) cell lines, with up to 12-fold IC\(_{50}\) variability. Interestingly, the Q61H mutant, known to exhibit the lowest intrinsic GTP-hydrolysis rates, is the most sensitive to MEK/ERK inhibition, suggesting that intrinsic biochemical properties of specific KRAS mutants affect drug response. Moreover, in our KRas\(^{\text{lox}}\) KRAS\(^{\text{MUT}}\) cell lines expressing specific KRAS mutants, we observed differential reactivation of upstream RTKs in response to MAPK inhibition, suggesting the existence of a functional crosstalk between specific KRAS mutations and upstream receptors that may be modulated by oncogenic signalling, with potential implications on drug resistance mechanisms.

**Conclusion**

- KRAS specific mutants retain unique GTP-hydrolysis features
- KRAS specific mutants have distinct growth properties in vitro and in vivo
- KRAS specific mutants show differential responsiveness to MEK/ERK inhibitors in vitro and in vivo.

**Senescence**

**PO-210** ANTI CANCER DRUG-INDUCED SENESCENCE IN GLOBLASTOMA CELLS IS ASSOCIATED WITH CHANGES IN DNA REPAIR CAPACITY

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**Introduction** Despite extensive research, malignant glioma remains the most aggressive and fatal type of brain tumour. Following resection, therapy is based on radiation concomitant with the methylating agent temozolomide (TMZ), followed by adjuvant high-dose TMZ treatment. The success of glioma therapy depends largely on the DNA repair capacity of the tumour cells, notable repair by MGMT, which confers resistance to TMZ. Beside DNA repair, other mechanisms involved in tumour protection may also play a role. These include the induction of a transient cell cycle arrest that provides time for repair and the induction of an irreversible cell cycle arrest in the form of senescence.

**Material and methods** MZ-induced senescence and repression of DNA repair was analysed in LN229, U87, LN308 and U138 glioblastoma cells using different techniques, including immunodetection, RT-qPCR, ChIP, Co-immunoprecipitation, ELISA, β-Gal staining, C\(_{12}\)FDG staining, AnnexinV/PI staining, flow cytometry, pharmacological inhibition and si-RNA mediated knockdown.

**Results and discussions** We show that following single and chronic TMZ exposure most glioma cells evade apoptosis and enter a senescent state. TMZ-induced senescence occurs in the G2/M phase of the cell cycle and is initiated by the ATR/CHK1-mediated degradation of CDC25c. It is further sustained by activation of p21 and NF-κB. Analysing the transcriptional regulation of DNA repair factors upon TMZ exposure, we found a strong repression of the mismatch repair (MMR) proteins MSH2, MSH6 and EXO1 as well as of Rad51, the central component of the homologous recombination pathway. The repression of these genes was regulated by the disruption of the E2F1/DP1 complex and is a specific trait of TMZ-induced senescent cells.

**Conclusion** Repression of DNA repair in senescent cells may result in acquired drug resistance to TMZ and could render these cells susceptible to the accumulation of additional genomic alterations induced by anticancer therapy. In case of an escape from senescence, these genetically altered cells could contribute to increased aggressiveness and therapy resistance in a recurring tumour.

**PO-211** PROFILING THE ALTERNATIVE SPlicing LANDSCAPE OF SENESCENT CELLS

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**Introduction** Cellular senescence, defined by an irreversible cell cycle arrest in response to potentially oncogenic stimuli, has been described as a protective mechanism in tumourigenesis and a therapeutic target in cancer. The senescence-associated secretory phenotype (SASP) is a pro-inflammatory response by senescent cells involving the release of cytokines, chemokines, growth factors and proteases that, in a cancer progression context, may be beneficial by the elimination of senescent cells or deleterious when triggering angiogenesis, cell proliferation and epithelial-to-mesenchymal transition. Despite senescence’s importance in cancer and the suggested role of alternative splicing in its regulation, the transcriptional heterogeneity of senescent cells has, to our knowledge, been extensively characterised only at the gene expression level.

**Material and methods** Next-generation sequencing of RNA (RNA-seq) allows alternative splicing quantification with unprecedented precision. The inclusion level of an exon is commonly quantified by its percent-spliced-in (PSI) value, i.e. the proportion of RNA-seq reads providing evidence supporting its inclusion. However, a PSI ratio does not incorporate information about the number of reads used in the quantification of the cognate alternative splicing event, directly proportional to the precision of its estimate. Beta distributions can be exploited in modelling inclusion levels, using reads supporting exon inclusion and exclusion as surrogates of the distribution’s shape parameters. We employed a computational