level of heterogeneity that included the same tumour subtypes also obtained using the ex vivo procedure.

Conclusion A set of the same genetic mutations is able to drive heterogeneous MM development suggesting that the specific phenotype of the resulting tumour depends on the specific cell-of-origin. Therefore, epigenetic differences rather than acquired mutations appear a determining factor for the subtype of MM that arises. This epigenetic state appears relative stability as interconversion of the tumour subtypes was not observed.

**PO-275 EFFECT OF IONISING RADIATION IN FADU CELL LINE - PRELIMINARY RESULTS**

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Introduction Head and neck cancer includes malignancies usually originated in upper aerodigestive tract mucosa. Despite hypopharyngeal cancer has a low incidence, patients have poor prognosis, advanced stage and distant metastatization. A low overall survival of these cancer might be explained by the presence of cancer stem cells (CSC). Identification of a CSCs markers panel could help to overcome this barrier allowing to develop target drugs. This study aims to evaluate and characterise the expression of proliferation, adhesion and CSC markers in a hypopharyngeal cell line (FaDu) before and after ionising radiation exposure.

Material and methods Paraffin cell blocks were performed in order to characterise by immunohistochemistry (IHC), epithelial characterisation (cytokeratin CAM 5.2, leukocyte common antigen (LCA), vimentin), tumoral aggressiveness (P16, P53, OCT4 and SALL4), proliferation (Ki-67) and CSC markers (EpCAM, CD10, CD44, CD117, CD133, beta-catenin). Also, 14 subtypes of high-risk HPV (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 16, and 18), EBV, Akt and Wnt expressions were analysed. Radiotherapy was performed with 0.5 × 10⁶ cells/mL, exposed to increasing doses of X-ray (0.5 to 10 Gy), except for control cells. Clonogenic assay and IHC were realised. Treated cells for IHC were fixed with alcohol 96%, 48 hour after treatment and posteriorly analysed the following antigens Ki-67, EpCAM, CD133, CD44, beta-catenin, CD117, CD10, Akt, Wnt, OCT4 and P53.

Results and discussions FaDu cell line showed positive immunostaining for cytokeratin, vimentin and negative to LCA. Relatively to tumoral aggressiveness, cells only expressed positively P16, while P53, OCT4 and SALL4 were negative. About proliferation it was observed Ki-67+. Between analysed stemness markers just CD44, CD133 and beta-catenin were expressed. Among HPV subtypes analysed only HPV18 was stained positive and EBV-. Adding, control cells expressed Akt + and Wnt + in normal conditions. Preliminary results related to ionising radiation effects showed that cell survival is X-ray dose dependent. Moreover, it was observed Wnt expression alterations with X-ray exposure.

Conclusion These results demonstrate that FaDu survival is affected by ionising radiation exposure. This may be associated with Wnt expression which is altered after irradiation, highlighting that this molecule is involved in cell fate determination, cell polarity, migration and cell proliferation.

**PO-276 PROGNOSTIC SIGNIFICANCE OF LGR5, AN INTESTINAL STEM CELL MARKER, IN COLORECTAL CANCERS. (RUNNING HEAD: LGR5 IN COLORECTAL CANCERS)**

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Introduction Leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5) has been suggested as a promising cancer stem cell marker in colorectal cancers (CRCs). However, the clinical significance of LGR5 expression in CRCs remains controversial. Here, we investigated the expression profile of LGR5 and determined the prognostic impact of LGR5 in a large cohort of CRC samples.

Material and methods LGR5 expression was determined with fresh-frozen CRC tissues by real time-PCR analysis. Tissue microarrays (TMAs) containing 1133 CRC samples were constructed, on which RNA in situ hybridization (ISH) for LGR5 was performed. The functional effects of LGR5 expression on cancer cell proliferation and migration was assessed by in vitro transfection technique.

Results and discussions LGR5 expression was higher in CRCs than in normal mucosa, and was not associated with other cancer stem cell markers. LGR5 positivity was observed in 43% of CRCs and positively correlated with old age, well to moderate differentiation, and nuclear β-catenin expression. Enhanced LGR5 expression remained during the adenoma-carcinoma transition, but substantially declined in the budding cells. Because nuclear β-catenin expression was consistently observed in the budding cells and TGFβ1 treatment or SNAIL overexpression did not result in a decrease in the LGR5 expression in LOVO cells, Wnt or EMT pathway are not likely to be responsible for LGR5 suppression. LGR5 expression showed negative correlations with microsatellite instability and CpG island methylator phenotype, and was not associated with KRAS and BRAF mutations, suggesting an implication of LGR5 in chromosomal instability (CIN) pathway in CRC carcinogenesis. Notably, LGR5 positivity was an independent prognostic marker for better prognosis. LGR5 overexpression led to reduced tumour growth by decreasing ERK phosphorylation along with decreased colony forming and migration abilities in DLD1 cells. Likewise, knockdown of LGR5 expression resulted in a decline in the colony forming and migration capacities in LOVO cells. These data suggest the suppressive role of LGR5 in CRC progression.

Conclusion Enhanced LGR5 expression remains persistent during the adenoma to carcinoma progression, and is associated with CIN pathway of colon carcinogenesis. Abrupt LGR5 down-regulation observed in the budding cancer cells at the invasive fronts is less likely due to altered Wnt or EMT signalling pathways. LGR5 was an independent prognostic marker for better clinical outcomes in CRCs.