Materials and Methods: To define the role of the KDELR-Golgi-SFK signalling cascade in cell invasion I assessed the ability of A375MM melanoma cells to degrade the extracellular matrix (ECM). Cells were transfected with different constructs to activate/inhibit KDELR and then plated on a fluorescent gelatin matrix in the presence of BB94, a broad spectrum metalloprotease inhibitor. After 16 hours, BB94 was washed out and cells were fixed after 3 hours and processed for immunofluorescence. The degradation areas, visible as dark "holes" in the fluorescent gelatin matrix, were quantified by the LSM510−3.2 software (Zeiss).
Results: The chronic activation of the KDELR by either over-expression of the KDELR itself, which induces its autoactivation, or transfection of artificial secretory proteins that act as KDELR agonists increases ECM degradation. In contrast, the over-expression of the KDELR mutant KDELR-D-193-N leads to a decrease in ECM degradation. The increase in ECM degradation correlates with an increase in SFK activation. Moreover, upon KDELR activation there is an increase in the phosphorylation levels of two SFK substrates, the ARF GTPase activating protein ASAP1 and the focal adhesion kinase FAK. These data indicate that KDELR-Golgi-SFK signalling affects ECM degradation through the involvement of ASAP1 and FAK.
Conclusions: We have investigated whether KDELR–Golgi–SFK signalling could regulate tumour cell invasion. Our data indicate that the chronic activation of this cascade promote ECM degradation. We believe that the Golgi complex has a crucial role in cell invasion, not only by providing hydrolytic enzymes for ECM degradation but also through KDELR-initiated-signalling. Our investigation is important because it highlights a new signalling cascade involved in the regulation of cell invasion thus expanding our knowledge of the metastatisation process and possibly providing new druggable targets to exploit for anti-metastasis therapy.

402 The endogenous EPO/EPOR system contributes to glioma cell proliferation both in vitro and in vivo

E. Pérès[1], S. Valable[1], J.S. Guillamo[1], J.F. Bernaudin[2], S. Roussel[1], M. Bernaudin[1], E. Petit[1]. [1]*UMR 6232 CI-NAPS, CERVOxy Team, Caen, France,* [2]*EA 3499, Service d'Histologie-Biologie Tumourale Hôpital Tenon, Paris, France*

Background: The biology of erythropoietin (EPO) has been recently re-evaluated following the discovery of its receptor (EPOR) on numerous cancer cells [1]. Concerning brain tumours, although the expression of the EPOR has been described on glioma cells [2], data from the literature remain descriptive and controversial [3] and to date no clear demonstration of a potential effect of EPO on controlling tumour growth has been described. Accordingly, the aim of this study was to evaluate and compare both in vitro and in vivo, the effect of two complementary strategies developed to block the biological effect of EPO/EPOR on glioma cell growth.
Material and Methods: Human U87 and U251 glioma cells were genetically modified by RNAi to stably invalidate EPO or EPOR expression. Full-length EPOR (9L-EPORF) or a truncated variant used as a negative dominant for EPOR (9L-EPORT) were stably overexpressed by rat 9L glioma cells. Effects of genetically modified cells were studied both in vitro on cell proliferation by automatic cell counting and in vivo by a longitudinal MRI follow-up of tumour growth and a survival study after intrastriatal implantation of these cells in Fischer rats (for 9L cells) or nude mice (for U87 and U251 cells).
Results: On one hand, the invalidation of either EPO expression (with shRNA EPO) or EPO availability (with soluble EPOR) on distinct human glioma cell lines led to a sustained decrease in cell proliferation from day 1 to day 4 (at day 4, U87-shEPO: 27±5% versus U87 control, $n = 3$, $p < 0.05$). On another hand, the invalidation of EPOR expression or EPOR signaling also induced a reduction of glioma cells number in vitro. Accordingly, in vivo, we observed on day 34 a decrease in tumour volume for the mice bearing U87-shEPOR ($59 \pm 16\ mm^3$), as compared to U87-Control mice ($107 \pm 30\ mm^3$; $n = 4$ for each group, $p = 0.05$). In addition, we also measured by MRI at day 13, a significant reduction of the tumour volume for animals bearing 9L-EPORT cells ($5.4 \pm 1.5\ mm^3$) in comparison to control rats (9L cells: $77.7 \pm 34.8\ mm^3$, $n = 4$ both groups, $p < 0.05$). An increase in the animal survival median was also observed for 9L-EPORT (28 days) in comparison to 9L rats (21 days, $n = 6$ both groups, $p < 0.05$).
Conclusions: Collectively, by the use of two strategies of EPO/EPOR inhibition on glioma cells, our results support that the autocrine EPO/EPOR loop on tumour cells might be a critical mediator of cell proliferation which might influence tumour outcomes of patients with brain tumours.
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403 Mitochondrial superoxide dismutase overexpression changes neuroendocrine, proliferation and apoptosis resistance features in prostate cancer cells

I. Quiros Gonzalez[1], R.M. Sainz[1], D. Hevia[2], M. Navarro Rego[1], A. Rodriguez Garcia[1], J.C. Mayo[1]. [1]*Instituto Oncologico del Principado de Asturias IUOPA, Morfologia y Biologia Celular. Universidad de Oviedo, Oviedo, Spain,* [2]*Instituto de Fermentaciones Industriales (IFI)-CSIC, Tecnologías Sectoriales, Madrid, Spain*

Prostate cancer (PC) is a tumour greatly associated with aging and oxidative stress one of the most accepted theories to explain aging. Therefore antioxidant enzymes such as MnSOD/SOD2 have been studied in relation to androgen-independent transition and resistance to radio/chemotherapy treatments. The main role of MnSOD is superoxide radical scavenging and thus this mitochondrial enzyme is induced, among other stimuli, as a radiation-adapted signal. Neuroendocrine (NE) cells are postmitotic cells and the less frequent cell type in the prostate gland that show androgen independence and resistance to treatment. Interestingly, NE differentiation is a common process along with PCa progression.
In order to study the role of MnSOD in NE cells, we stably overexpresed androgen dependent LNCaP. In these clones we first confirmed the presence of NE markers and apoptotic-related proteins by western blotting. Then we tested (1) proliferation rate; (2) the ability of cells to grow in androgen deprived medium both by cell counting; (3) apoptosis resistance to chemotherapeutic treatment by MTT viability assay; and (4) the potential capacity of MnSOD-expressing cells-conditioned media of inducing cell growth in androgen independent PC-3 cells by DNA staining with Hoechst.
MnSOD overexpressing LNCaP clones, namely MnSOD-S4 and S12, show typical NE morphology when compared to mock clones (pcDNA-P2), which was also confirmed by levels of Synaptophisin, a widely used NE marker. Surprisingly, a higher proliferation rate was observed in MnSOD-S4 and S12 clones, indicating that MnSOD overexpression keeps proliferation capacity. In androgen-stripped media, MnSOD-S12 clone displays a significantly higher proliferation rate than pcDNA-P2 while protein levels of androgen receptor are decreased in both, MnSOD-S4 and S12 clones. Regarding apoptosis resistance, MnSOD-S12 clone showed higher resistance etoposide-induced cell death Finally, PC-3 cells maintained with conditioned medium obtained from MnSOD-S4 showed higher levels of proliferation compared with pcDNA-P2.
The increase of MnSOD expression is enough to induce most of the reported morphological and biochemical characteristics of androgen-independent NE-like cells in PC. This would indicate that redox balance mediated by MnSOD could be a key step in androgen dependent-independent transition in PC.

404 A novel role for JAM-A as a crucial regulator of breast cancer cell motility through downstream effects on Rap1GTPase and Beta1-integrin

E. McSherry[1], A.M. Hopkins[1], A.D.K. Hill[1]. [1]*Royal College of Surgeons in Ireland, Surgery, Dublin, Ireland*

Introduction: The cell-cell adhesion protein junction adhesion molecule-A (JAM-A) influences epithelial cell morphology and migration. We have previously demonstrated that high JAM-A levels in breast cancer tumour cells are significantly associated with poor prognosis in breast cancer patients (McSherry, 2009). In addition, we have recently shown that functional inhibition or knockdown of JAM-A decreases MCF-7 breast cancer cell migration, likely due to reduced levels of β1-integrin. The aim of our ongoing studies is to define the pro-migratory signalling cascades at play in breast cancer cells from JAM-A (at the cell-cell interface) to β1-integrin (at the cell-matrix interface).
Materials and Methods: MCF7 cells following siRNA-mediated JAM-A gene knockdown (JAM-A KD cells) were investigated via western blot and immunoprecipitation analysis to determine protein expression of putative signalling pathway proteins (downstream of JAM-A). In addition, JAM-A KD cells were assayed for alterations in cancer cell migration and cell adhesion. As Rap1 is a known activator of integrins, cell migration of MCF7 cells was determined following treatment with either a Rap1GTPase pharmacological inhibitor or a b1-integrin inhibitory antibody. Analysis of active Rap1 was performed in MCF7 cells following either JAM-A gene knockdown or JAM-A protein antagonism. Affects of JAM-A antagonism on PDZGEF2 (a crucial Rap1 activator) was assessed by immunofluorescence and western blot analysis.
Results: We have demonstrated that following JAM-A gene knockdown the protein expression of Rap1 and the Rap1 regulator AF-6 is decreased, and direct association of JAM-A and AF-6 is altered. In addition, the activity of Rap1 is reduced upon either JAM-A knockdown or inhibition. Furthermore, we demonstrated that breast cancer cell migration is decreased upon inhibition of both Rap1GTPase and b1-integrin. Finally, we demonstrate that PDZGEF2 localisation is altered following JAM-A inhibition.
Conclusion: We suggest that JAM-A over-expression can initiate a signalling cascade involving AF-6, PDZGEF2, Rap1GTPase and b1-integrin, to promote