metastasis development using murine melanoma metastasis model establish from our stable cell lines.

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RhoB controls estrogen receptor target genes expression through a modulation of ER recruitment on the promoter binding sites, in MCF-7 cells

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Around two thirds of mammary tumors express estrogen receptors (ER) and hormonotherapy is then recommended. Nevertheless, resistances to these treatments systematically occur and impose the search for new pharmacological targets. Estrogens act mainly through the well-known ERα but cross-talks have been clearly demonstrated between ERα and growth factors signalling pathways. Ras family proteins, such as Rho prenylated proteins, are key elements in those cross-talks. Indeed, we and others previously showed that prenylated proteins stimulate the proliferation of MCF-7 cells (hormonodependent breast cancer cell line) and on the contrary negatively regulate transcriptional responses mediated by ER. The purpose of this study was to analyze the effect of estrogens on RhoB expression and activation, and conversely, the effect of RhoB on ER expression, on its target genes expression and on ER recruitment on the promotor of target genes (progesteron receptor and pS2). We first showed that a significant increase of the active GTP bound form of RhoB is observed after 30 minutes of estrogen stimulation with no modification of RhoB expression at this stage. To decipher the mechanisms involved in the effects of RhoB on ER-mediated activities, we abolished the expression of RhoB using two sequences of interfering RNA in MCF-7 cells. On the one hand, we demonstrated that RhoB extinction significantly decreases ER protein and mRNA expression (confirmed in RhoB-/- mice). On the other hand, RhoB extinction clearly diminishes the expression of a luciferase reporter gene controlled by the vitellogenine Estrogen Responsive Element (MELN cells). Similarly, RhoB extinction decreases the progesteron receptor and pS2 mRNA levels. To explain these effects, we analyzed ERα recruitment on the Estrogen Responsive Element or ER binding site of each of these 3 genes, and demonstrated that RhoB extinction increases ERα recruitment to the pS2 and vitellogenine genes, and on the contrary, decreases it to progesteron receptor. In brief, our results evidence RhoB participation in the balance of recruitment of ER to its various target genes, individually modulating their expression. Further investigations, especially studies on hormonoresistant breast cancer cells are now ongoing for a better understanding of hormone resistance mechanisms.

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CXCR4 expression mediates the survival and proliferation of glioma cells

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Malignant gliomas are brain neoplasms that account for more than 50% of the tumours that arise within the central nervous system. They are higly proliferative, angiogenic and locally very invasive. The mechanism by which malignant glioma grow is still not understood but some evidences suggest the involvement of chemokines.

Chemokines are a family of molecules that regulate chemotaxis of leukocytes into tissues, promote mitosis and cell survival. The family of the CXC chemokines, and in particularly the CXCL12 chemokine and its receptor CXCR4, has been associated with cell proliferation and cell survival of several tumours.

To better understand the role of CXCL12/CXCR4 in malignant glioma we studied the expression of CXCR4 in a glioma cell line, the U-118 cell line. We also determined the contribution of the CXCR4 to cell adhesion, proliferation, survival and migration. The assays were performed in the presence of CXCL12 with or without AMD3100. CXCR4 expression was evaluated by western blot and immunofluorescence. To determine whether CXCR4 was functionally active, the activation of Akt was evaluated by western blot. Cell adhesion was measured under static conditions. Cell proliferation was determined using BrdUrd incorporation. Cell survival was addressed using two stains hoescht and propidium iodide. Cell migration assays were carried out using migration chambers.

Our results showed that CXCR4 is expressed in the U-118 cell line. In the presence of CXCL12 an increased adhesiveness of cells to the collagen matrix was observed. In addition, CXCL12 significantly increases the cell proliferation and survival. The CXCR4 antagonist, AMD3100, induces a significant reduction of cell proliferation and a significant increase in the number of apoptotic cells. Furthermore, in the presence of CXCL12, activation of Akt by CXCL12, the survival kinase, was also observed. The chemotaxis assay revealed that CXCL12 was chemotactic and induced the migration of glioma cells, indicating that CXCR4 expression is required in the invasion of glioma cells.

In conclusion, our in vitro studies, using the U-118 cell line, indicate that CXCR4/CXCL12 is implicated in the modulation of glioma cell proliferation, survival and migration.

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CDK-dependent phosphorylation of Bim during Taxol-induced cell death

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The mitochondrion plays an important regulatory role during caspase-dependent and caspase-independent cell death, through the release of apoptogenic proteins such as cytochrome C, Smac/Diablo, AIF, Omi/Htra and Endonuclease G from the intermembrane space. Mitochondrial release of apoptogenic proteins is regulated by the Bcl-2 protein family that is made up of both pro-apoptotic and anti-apoptotic members. Post-translational modification of Bcl-2 protein family members, such as phosphorylation and proteolytic cleavage, plays an important part in regulating their activity.[1]

The BH3-only pro-apoptotic family member, Bim, is phosphorylated by the Erk and JNK MAP kinases. Erk phosphorylates Bim resulting in proteasomal degradation of Bim.[2] The JNK MAP kinase phosphorylates Bim directly on serine and threonine residues resulting in its release from microtubules. Furthermore, JNK induces upregulation of Bim through the activation of the transcription factor, c-jun.[3,4]

It has been previously shown that chronic myeloid leukaemia (CML) cells undergo caspase-independent cell death following disruption of the microtubule network by microtubule targeting agents including Taxol.[1 and unpublished results] In this study it has been found that Bim resides in the mitochondria of CML cells. In addition, the two Bim isoforms, Bim EL and L, undergo phosphorylation following treatment with Taxol. Phosphorylation of Bim occurs in a time- and dose-dependent manner and precedes Taxol-induced cell death in CML cells. On further examination it has been found that phosphorylation of Bim EL occurs within 8 hours treatment with Taxol, whereas phosphorylation of Bim L does not occur until 12 hours after treatment. Synchronisation of K562 CML cells by double thymidine block and treatment with Taxol, has revealed that phosphorylation of Bim correlates with the accumulation of cells in G2/M.Pre-treatment of cells with the CDK inhibitors, Flavopiridol and Roscovitine, was found to block the phosphorylation of Bim EL and L upon Taxol treatment.

These results suggest that phosphorylation of Bim at the mitochondrion occurs during mitosis, which may represent an important event that connects cell cycle arrest to the cell death machinery following microtubule disruption.

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Targeting membrane rafts inhibits protein kinase b by disrupting calcium homeostasis and attenuates malignant properties of melanoma cells

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Failure of current therapeutic modalities to treat melanoma remains a challenge in clinical and experimental oncology. The aggressive growth and apoptotic resistance of melanoma are mediated, in part, by aberrantly activated protein kinase B/Akt (PKB). In many cells, PKB signaling depends on integrity of cholesterol-enriched raft micro-domains; however, it is still unclear if rafts support PKB deregulation in melanoma cells. The ablation of rafts in murine (B16BL6-8, JB/RH1) and human (GA) melanoma lines by cholesterol-chelating methyl-beta-cyclodextrin (MCD) efficiently reduced levels of active PKB in a dose- and time-dependent manner, while reconstitution of rafts restored PKB activity. PKB was also sensitive to the membrane permeable Ca2+ chelator (BAPTA-AM) and calmodulin inhibitor