between VEGF and S1P signalling in the migration and proliferation of ML-1 cells.

We now show that S1P signalling regulates VEGFR-2 protein expression. In ML-1 cells transduced with a dominant negative sphingosine kinase (SK^{G82D}), the VEGFR-2 protein expression is slightly lower than in Mock transduced cells. Over-expression of SK (SK^{WT}) induces a higher protein expression than found in Mock transduced cells. However, this up-regulation in receptor expression is not mediated by transcriptional regulation.

Preliminary data suggests that in ML-1 cells S1P-receptors (S1P1-3, 5) form complexes with VEGFR-2, both in the presence and absence of serum. Interestingly, VEGFR-2 protein expression is regulated by ERK1/2 in SK^{WT} expressing cells as well as in native ML-1 cells. Inhibiting VEGFR-2 in native ML-1 cells also inhibited S1P-induced ERK1/2 phosphorylation. Similar results were found in SK^{WT}-expressing cells.

We have previously shown that through the activation of novel and classical isoforms of PKCs (i.e. PKC-α) and subsequent activation of SK, ERK1/2 may be phosphorylated, resulting in the induction of migration. Interestingly, ML-1 cells display at least two migratory pathways, differing in their sensitivity to VEGFR-2 inhibition. We have previously shown that S1P-induced migration is sensitive to VEGFR-2 inhibition; however, an OAG induced migratory response is insensitive to inhibition of VEGFR-2, indicating that a receptor complex may indeed mediate the S1P-induced migration.

ML-1 cells secrete substantial amounts of VEGF-A, which can be stimulated by micromolar concentrations of S1P. Although, transducing ML-1 cells with SK^{G82D} or SK^{WT} did not affect basal VEGF-A secretion, inhibiting ERK1/2 significantly reduced the VEGF-A secretion of SK^{WT}-cells as well as of Mock-transduced cells. We conclude that ERK1/2 plays a major role in the cross-talk between the signalling pathways of S1P- and VEGF-receptors. Taken together ERK1/2 regulates both the expression of VEGFR-2 and VEGF-A secretion in SK^{WT} cells.

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Significant down-regulation of DNA repair systems in non-small cell lung tumours that reactivate telomerase

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Telomere function and DNA damage response pathways are frequently inactivated in cancer. Moreover, some telomere-binding proteins have been implicated in DNA repair. The main aim of this work consists of evaluating possible relationships between telomere dysfunction and DNA repair systems in non-small cell lung cancer (NSCLC).

We analysed 83 NSCLCs and their corresponding control samples obtained from patients submitted to surgery. Telomere function was evaluated by determining telomerase activity and telomere length. DNA repair expression assays were established by using cDNA arrays containing 96 DNA-repair genes and by Real Time Quantitative PCR.

Our data indicated that 83.13% of tumours showed telomerase activity. We observed significant associations between enzyme activity and TNM stage (P = 0.008), size (P = 0.041) and histology of tumours (P = 0.001). Also our results revealed that shorter telomeres were significantly associated with tumours that had grown into the area of mediastinum or cancers with a malignant pleural effusion (P = 0.003). In relation to expression assays, we detected a group of DNA repair genes whose expression levels were significantly associated with telomerase activity. As expected, TERT expression (P = 0.044) was significantly increased in the group of tumours displaying telomerase activity. However, expression data for DCLRE1C (P = 0.001), GTF2H1 (P = 0.009), PARP3 (P = 0.005) and MLH1 (P = 0.003) indicated a significant down regulation in association with telomerase activity. Moreover, TRF2 was down regulated in telomerase positive tumours showing significant telomere shortening (P = 0.042).

In conclusion, results here presented suggest an association between the loss of several DNA repair genes and telomerase activity, which may be of relevance in the pathogenesis of non-small cell lung cancer.

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MKP1 regulates susceptibility to genotoxic stress

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Dual-specificity phosphatase type 1, DUSP1/MKP1, is a member of the dual-specific family of phosphatases that dephosphorylates MAPKs, including ERK, JNK and P38. MKP1 is a nuclear protein, whose basal levels are low in unstressed or unstimulated cells and its expression is induced following stimulation with mitogens, oxidative stress, hypoxia, and DNA damaging agents. Additionally, different studies have shown that MKP1 is overexpressed in different types of cancer (breast, ovarian, prostate and lung carcinoma). Many chemotherapeutic drugs induce apoptosis in cancer cells as a consequence of activation of JNK and p38 pathway. The ability of MKP1 to decrease the activity of these kinases results in protection from apoptosis and drug resistance. Resistance to radiation and chemotherapy is one or the major obstacles in cancer treatment. Thus, interference of MKP1 may be an alternative strategy for manipulating MAPK pathways in a cell-type specific manner. Indeed, previous work in our group has shown that inhibition of MKP1 expression sensitizes non-small-cell lung cancer (NSCLC) to cisplatin.

In this work, we have investigated the role of MKP1 in modulating antitumoral-induced apoptosis. Mouse embryonic fibroblasts (MEFs) derived from wild-type (MEF+/+) and MKP1 knock-out (MEF-/-) mice were exposed to different drugs commonly used in the clinic. Cell viability was studied by crystal violet staining method; MAPK activity, c-jun, caspase-3 and MKP1 expression levels were determined by inmunoblotting, using specific antibodies. MEF+/+ cells treated with alkylating agents showed a direct correlation between MKP1 expression and JNK or p38 inactivation, and in turn a lower sensitivity to drugs compared to MEF-/-. In addition JNK and p38 activity was strongly activated in MEF-/- and the cells were hypersensitive to these drugs. On the other hand, no differences were observed in either sensitivity or MAPK activity between MEF+/+ or MEF-/- after treatment with agents that induce double strand breaks; either agents targeting cytoskeleton; or drugs blocking DNA synthesis, which are not able to induce MKP1 expression.

Our results strongly suggest that MKP1 specifically regulates survival in response to alkylating agents by modulating JNK and p38 activity implicating MKP1 as an important mediator of chemoresistance. Therefore, pharmacological inhibition of MKP1 could be used in combination with alkylating drugs to induce chemosensitization and overcome chemoresistance.

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SK3 channel promotes melanoma cell migration

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Numerous studies have demonstrated that potassium channels interfere with pathways controlling the balance between cell growth and cell death. In contrast, the role of potassium channels in tumour cell dissemination and metastasis has been less intensively investigated. Among potassium channels we recently found that SK3 channel, a member of apamin-sensitive small-conductance calcium activated potassium channels (SK_{Ca}), is a mediator of breast cancer cell migration[1]. Since melanoma is an extremely aggressive disease with metastatic potential, we investigated if SK3 channel is expressed in melanoma cell lines and if this channel plays a role in melanoma cell migration.

To investigate the presence of SK3 channel in melanoma cells we first performed RT-PCR and Western blot analyses in three human melanoma cell lines, SKmel28, Bris and 518A2. We found that SK3 gene expression and proteins were detected in Bris and 518A2 but not in SKmel28 cells. Then, using apamin a specific blocker of SKCa channels, we compared the migration behaviour of melanoma cell expressing or not SK3 protein. This blocker reduced migration of Bris and 518A2 cells but didn't affected migration of SKmel28 cells. Consequently, apamin reduced migration only in cells expressing SK3 protein. To fully demonstrate the contribution of SK3 channel in melanoma cell migration we have enforced SK3 gene expression in SKmel28 cells and knocked-down SK3 transcripts in Bris and 518A2 cells using lentiviral vector containing respectively a SK3 cDNA and a shRNA-SK3. Western blot experiments confirmed a large decrease of SK3 protein in Bris and 518A2 cells and a SK3 protein expression in SKmel28 cells. Patch-clamp recordings demonstrated that if silencing SK3 expression depolarised plasma membrane of Bris and 518A2 cells, stable expression of SK3 protein hyperpolarised membrane potential of SKmel28 cells. In parallel, we found that expression of SK3 gene in SKmel28 cells increased their migration and depletion of SK3 gene in 518A2 and Bris cells decreased their migration. In contrast to numerous potassium channels, in our case, SK3 channel seems to not interfere with cell proliferation or cell death.

In conclusion and as observed for breast cancer cells, SK3 channel is a mediator of melanoma cell migration. Moreover, these new results suggested that SK3 channels promote cancer cell migration by hyperpolarising plasma membrane leading probably to subsequent Ca^{2+} influx. To go further we will study SK3 involvement in tumour growth and/or

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

[1] Potier et al. (2006) Identification of SK3 channel as a new mediator of breast cancer cell migration. Mol Cancer Ther; 5(11) p2946-2953

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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