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

C. Frías[1], P. Ortega[1], A. Morán[1], C. De Juan[1], T. Fernández-Marcelo[1], A. Gómez[2], F. Hernando[2], A.J. Torres[2], M. Benito[1], P. Iniesta[1]
[1]Facultad de Farmacia, Universidad Complutense, Bioquímica y Biología Molecular II, Madrid, Spain; [2] Hospital Clínico San Carlos, Servicio de Cirugía II, Madrid, Spain

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

V. Rodriguez Fanjul[1], I. Sanchez-Perez[1], R. Perona Abellon[1]
[1]Biomedical Research Institute, Departamento de Modelos Experimentales de Enfermedades Humanas, Madrid, Spain

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

A. Chantome[1], A. Girault[1], M. Potier[1], P. Vaudin[2], C. Collin[2], M.L. Jourdan[1], J.C. Pagès[2], P. Bougnoux[1], C. Vandier[1], V. Joulin[3]
[1]INSERM U921, Université François Rabelais, Nutrition Growth and Cancer, Tours, France; [2] ERI19 INSERM EA3856, Université François Rabelais, Virus Pseudovirus: Morphogenesis and Antigenicity, Tours, France; [3] Institut Gustave Roussy, Genome and Cancer, Villejuif, France

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