322. Treatment options and tumour biology of malignant pleural mesothelioma

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Antitumor activity of MEK and PI3K inhibitors in malignant pleural mesothelioma

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Background: Malignant pleural mesothelioma (MPM) is an aggressive malignancy, and there is no approved targeted therapy for this disease.
Objective: We investigated the role of mitogen-activated protein kinase kinase (MEK) inhibitor and phosphatidylinositol 3-kinase (PI3K) inhibitor as targeted therapies for MPM.
Method: We examined the therapeutic efficacy of the MEK or PI3K inhibitor against human MPM cell line EHMES-10 both in vitro and orthotopically inoculated into severe combined immunodeficient (SCID) mice. In addition, the molecular mechanisms of these agents were confirmed *in vitro* and *in vivo* experiments.
Results: MEK or PI3K inhibitor suppressed the growth of MPM model in dose dependent manner both *in vitro* and *in vivo* studies. In addition, combining MEK inhibitor with PI3K inhibitor resulted in an additive growth inhibitory effect. EHMES-10 cells showed increasing the G1 cell cycle arrest and apoptosis by treatment of MEK or PI3K inhibitor *in vitro*. Western blot analysis *in vitro* and *in vivo* study showed increasing the $p27^{kip1}$ and cleaved PARP expression and decreasing the Cyclin E, CyclinD1 and procaspase 3 expressions. In addition, these agents decreased the expression of hypoxia-inducible factor 1α and vascular endothelial growth factor, which play an essential role in tumor angiogenesis and progression.
Conclusion: Our results suggest that MEK or PI3K inhibitor is a promising therapeutic strategy, and also provide a basis for useful combination of MEK and PI3K inhibitors in patients with MPM.

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mTOR inhibition blocks tumor growth and pleural fluid accumulation in experimental murine mesothelioma

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mTOR is up-regulated in malignant mesothelioma. We aimed to evaluate the effect of Temsirolimus, an mTOR inhibitor, in in vivo models of the disease.
AE17 and AB1 murine mesothelioma cells were injected into the right flank of syngeneic mice (C57BL/6 and BALB-c, respectively) to create subcutaneous tumors. C57BL/6 mice were injected intrapleurally with AE17 cells to create pleural tumors and effusions. Animals were treated with Temsirolimus (20mg/kg) or vehicle, 5 days/week starting when tumors become palpable (flank model) or on days 2-6, 9-15 following the intrapleural injection of tumor cells (pleural model). Among mice with AE17 flank tumors, the mean ±SEM tumor volume at day 26 was 1261±383mm^3 in control and 383±86mm^3 in treated animals ($p<0.001$). Tumor cell apoptosis, assessed by TUNEL was significantly enhanced in mice treated with Temsirolimus ($p<0.001$). In the AB1 flank model, tumor volume was 1197±253mm^3 in control and 174±76 mm^3 in treated animals ($p=0.026$). Among mice with pleural AE17 tumors, the mean ±SEM pleural fluid volume at day 15 was 532±119microL in control and 240±44microL in treated animals ($p=0.018$). The mean ±SEM pleural tumor weight was 739±72mg in control

and 256±10mg in treated animals ($p<0.001$). Additionally, Temsirolimus retarded murine mesothelioma cell growth and reduce the phosphorylation/activation of the mTOR downstream protein, p70S6K protein, in vitro.
Inhibition of mTOR substantially reduced syngeneic mesothelioma growth blocked pleural fluid accumulation in animals bearing mesothelioma tumors.

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Epigenetic deregulated translation control of C/EBP-alpha leads to increased mesothelioma cell proliferation

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Malignant pleural mesothelioma (MM) resists all available anticancer therapies. A major pathology of MM is the uncontrolled cell proliferation and the fast local spreading with rare metastasis. Therefore the inhibition of proliferation is a major therapeutic target. Proliferation of MM cells was linked to mitogen activated protein kinase (MAPK) activity. In this study we characterised the regulation of MAPK regulated CCAAT/Enhancer binding proteins (C/EBP) and their role in MM cell proliferation. In five human MM cell lines, cytosolic and nuclear protein expression was determined by immuno-blotting and immuno-chemistry in tissue sections. Transcription of C/EBPs was determined by real time PCR and translation by a translation reporter assay. We observed a cell compartment specific expression pattern of p38-α, -β and -γ MAPK in MM cells. Erk1/2 and p38 MAPK together up-regulated the expression of C/EBP-β and -δ, while C/EBP-α was not expressed. Compared to mesothelial cells C/EBP-α translation was reduced in MM, while the mRNA was constitutively expressed. MM cells expressed a relative high level of the C/EBP-α translation suppressor calreticulin, while eIF4E was not significantly modified. Cell proliferation was inhibited by either the blockade of Erk1/2, or p38-β and -γ MAPK, or C/EBP-β. Transfection with a C/EBP-α expression vector reduced proliferation and increased the MM cell's sensitivity to steroids. Our data implies that in human MM cells an epigenetic mechanism deregulates the translation control of the cell differentiation factor C/EBP-α which leads to increased proliferation and drug resistance.

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The regulatory effect of microRNAs on STAT signaling in malignant mesothelioma

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Background/Aims: STAT1 is up- and STAT3 is downregulated in human malignant mesothelioma (MM), a cancer with little knowledge about predictive factors of outcome. The negative feedback loop of STAT1 is not functioning: SOCS1 and PIAS1 are downregulated in MM. MicroRNAs (miRNAs) regulate the expression of target mRNA. Therefore we aimed to quantify selected miRNAs in MM which are thought to be involved in the regulation of the STAT signaling pathway.
Material/Methods: RNA was obtained from 35 formalin-fixed and paraffin-embedded tumor tissue samples. MiRNAs were selected via *in silico* target prediction tools. Quantitative real-time PCR was used to assess miRNA expression levels. The reference gene RNU6B was used for normalization. An immunohistochemical (IHC) staining with 5 antibodies was performed on tissue microarray sections to correlate it with the results of the miRNA detection.
Results: MiR-106a (targeting STAT3) expression was increased in 63% of cases. MiR-155, miR-19a and miR-30d* (targeting SOCS1, SOCS1 and STAT1, respectively) were downregulated in all cases. Due to very low expression levels, miR-196a*, miR-608 and miR-765 (targeting SOCS6, PIAS1, SOCS3 respectively) were not detected. Positive IHC staining was achieved for STAT1, pSTAT1(Ser727), STAT3 and PIAS1. STAT1 was higher expressed than STAT3; SOCS1 was not detected by IHC.
Conclusion: The inverse correlation between pSTAT1 and miR-30d* (p=0.014) indicates a regulatory effect and this miRNA may interact with STAT1 (p=0.062). STAT3 is not affected by miR-106a (p=0.53) although this miRNA is expected to play an important role in MM. Therefore additional targets have been selected which are currently investigated.

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Epithelial-to-mesenchymal transition in malignant mesothelioma

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Epithelial-to-mesenchymal transition EMT is a molecular-cellular process activated during embryonic development and tissue remodelling, by which epithelial cells lose their polarity and cell contacts, acquire the expression of mesenchymal markers and manifest a migratory phenotype. The progressive loss of E-cadherin is coupled with expression of non-epithelial cadherins, process known as "cadherin switching". As tumours often mimic embryonic development, it has been postulated that EMT represents a transient event in carcinomas progession. Malignant Mesothelioma MM could represent an EMT *in vivo* model, because tumor cells can exhibit epithelial, sarcomatous and biphasic differentiation. Forty five patients with MM were investigated by immunohistochemical expression of cadherins E,N,P,11,p120 catenin,SPARC and caveolin in two tissue microarrays. Protein expression was scored from 0 to 3 in tumour and stroma. Data were correlated with histologic patterns, thoracoscopy findings and survival. E-P cadherins expression was observed in 79,3% of epithelial MM without evidence in mesenchymal component of mixed and sarcomatous types. N-11cadherins were detected in 20,6%, 29.4% and 17.6% of these histotypes,respectively.The mesenchymal markers were detected in 100% of sarcomatous and mixed MM and in a many samples of epithelial group. Immunohistochemical data correlated with metastatic status, multi-focal disease and poor survival, showed, in epithelial MM forms, weak or absent E-Pcadherins expression, while N-11cadherins, mesenchymal markers and P120 catenin were observed. Our results suggest that the aggressiveness of MM,could be explained by the acquisition of a mesenchymal phenotype in the context of EMT

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WITHDRAWN

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Multimodality treatment of malignant pleural mesothelioma

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There is no widely accepted standart of care for patients with malignant pleural mesothelioma (MPM). Multimodality treatment protocol should be the standard approach in suitable patients and performed as a part of a trial: Biopsy proven MPM of non-sarcomatiod cell type, T1-3, N0-1, M0, patient fit for extrapleural pneumonectomy (EPP), neo-/adjuant chemotherapy, and radical hemithoracic irridation.
In this study we evaluated the outcome of our patients with MPM who were treated by multimodality schedule including EPP, radical hemithoracic irridation, and cisplatin-pemetrexed/gemcitabine chemotherapy regimen.
A total of 29 patients who consecutively underwent multimodality treatment schedule, 15 men, 14 women, were included. Of the patients 24 had epithelial cell type, 5 were mixed. 12 patients had stage 1 disease, 6 had II, 10 had III, 1 had IV (after surgery). Perioperative mortality (in 1 month) was 14% (4/28), mortality during multimodal therapy schedule was 18% (5/28). Patients completed multimodal Schedule were 19 (68%). Of the 19 patients completed multi-modality treatment, 11 died, 8 are alive.

For all patients, 28 cases, median survival was 19 months. For 19 cases completed multimodality treatment schedule, MS was 41 months. The rate for 12, 36, 60-month survival were 89.5%, 42.1%, 31.6% respectively. For 9 cases who could not be completed multimodality tretament Schedule, median survival was 4 months. We concluded that multimodality treatment schedule in MPM is provided quite longer survival for selected cases.