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RESEARCH HIGHLIGHT

# Targeting receptor tyrosine kinases in malignant pleural mesothelioma: Focus on FGF-receptors

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**Fibroblast growth factor receptors (FGFRs) constitute a subfamily of receptor tyrosine kinases. Four different receptors, FGFR1-4, bind 18 different fibroblast growth factors (FGFs) and signal mainly along the mitogen-activated protein kinase (MAPK), the phosphatidylinositol 3 kinase (PI3K) and the phospholipase c gamma (PLCγ) pathway. Physiologically, they are major regulators of embryonic development and metabolism. Deregulation of FGFR signals is increasingly recognized to play important roles in malignant diseases and may constitute a feasible therapeutic target. We recently investigated their role in malignant pleural mesothelioma (MPM), an aggressive malignancy mainly caused by asbestos exposure and with currently limited therapeutic options. We demonstrated high expression of several FGFs/FGFRs, especially FGFR1, FGF2 and FGF18 in cultured tumor cells and tissue specimens and identified FGFR-mediated signals as major driver of MPM cell growth, survival and migration. FGFR blockade by a tyrosine kinase inhibitor or by a dominant-negative receptor construct resulted in reduced MPM growth *in vitro* and *in vivo* and, furthermore, enhanced the efficacy of chemo- or radiotherapy. Several other receptor tyrosine kinases, including EGFR, MET and AXL were found to be overexpressed in MPM but translation into clinically successful therapeutic approaches has not yet been achieved. Inhibition of FGF-receptors may have the advantage of targeting both the tumor cells as well as the tumor vasculature and should be further evaluated.**

***Keywords:*** FGF, FGFR, malignant pleural mesothelioma, receptor tyrosine kinase, targeted therapy

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Malignant pleural mesothelioma (MPM) is an aggressive malignancy affecting the pleural linings. It is primarily caused by asbestos exposure and the resulting chronic inflammation in the pleural cavity. Due to the widespread use of asbestos in the past, the long latency period of 20 - 40 years and the ongoing mining and use of asbestos in some countries, the incidence of MPM is expected to increase markedly over the next decades [1].

Fibroblast growth factors (FGFs) and their high-affinity receptors (FGFRs) are a highly conserved family of 18 specific ligands and 4 receptor tyrosine kinases located on the cell surface [2]. Activation by ligand binding results in receptor dimerization and phosphorylation followed by signal transmission to distinct downstream pathways including the mitogen-activated protein kinase (MAPK), the phosphatidylinositol 3 kinase (PI3K), and the phospholipase c gamma (PLCγ) pathways. These signaling cascades regulate crucial mechanisms such as cell proliferation, survival, migration, invasion or chemoresistance [3].

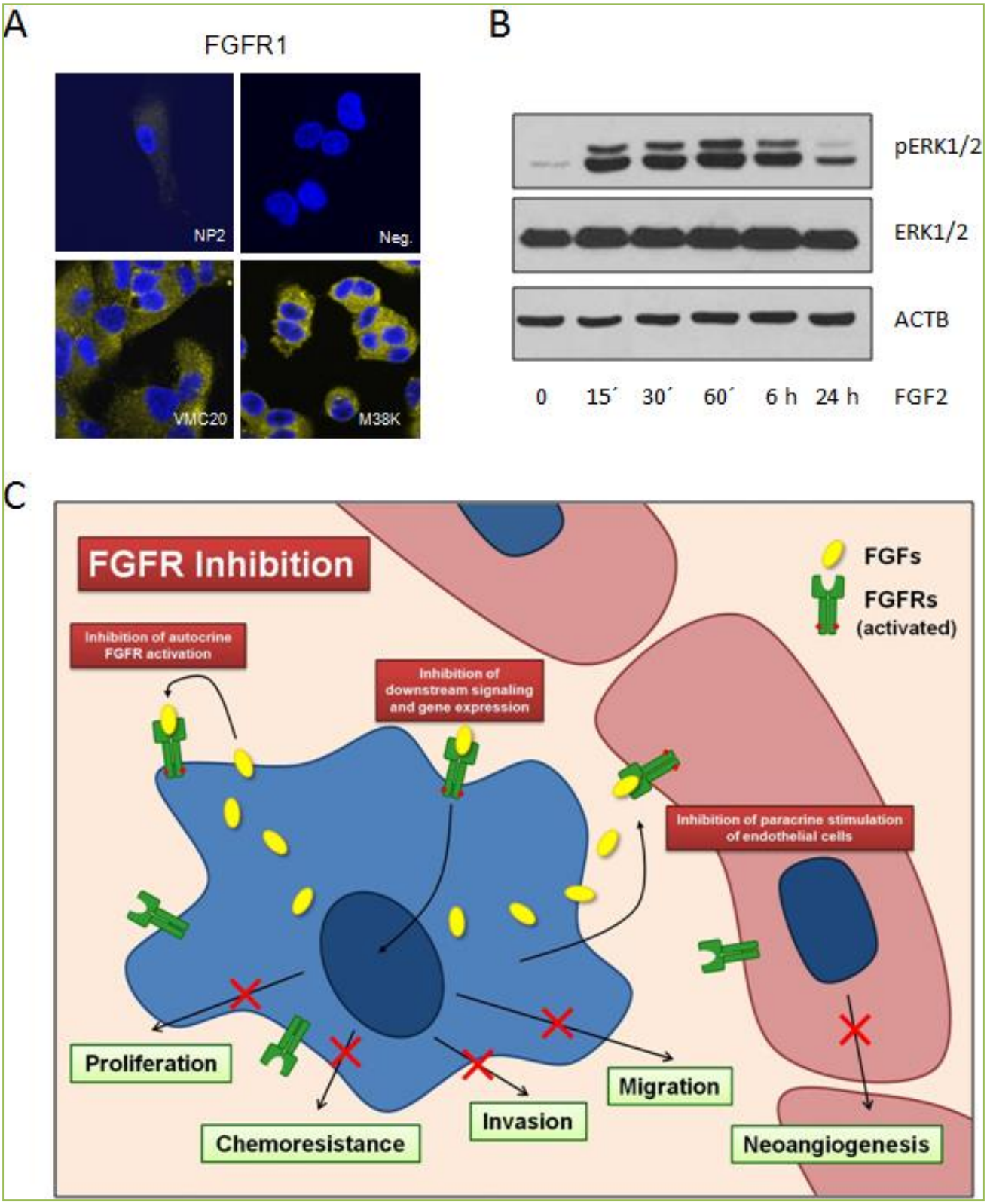


**Figure 1. Rationale for FGFR targeting in malignant pleural mesothelioma.** (A) Immunofluorescence images showing FGFR1 expression in normal mesothelial cells (NP2), as well as epithelioid (VMC20) and biphasic (M38K) MPM cells. Neg. = negative control with pre-immune serum instead of primary antibody. (B) Immunoblot analysis showing ERK phosphorylation at different time points after stimulation with recombinant FGF2 (10 ng/ml) in SPC212 cells. Methods for A and B are described in Schelch et al. 2014 [8]. (C) Schematic representation of the roles of FGF signals and effects of their inhibition in an MPM tumor cell (blue) and the microenvironment (endothelial cells, red).

The FGF axis has important physiological roles during organogenesis and embryonic development, and defects or alterations of FGF signals are associated with several kinds of disease, most notably with skeletal malformations caused by activating mutations in FGFR1 or FGFR3 [4]. Another significant feature of FGF signals is the promotion of angiogenesis in wound healing by stimulating proliferation and migration of endothelial cells [5]. Hyperactivation by mutations, amplifications or translocations can function as a major driver of tumor progression in a variety of malignancies, for instance in bladder and cervix carcinomas and multiple myeloma [6-7].

In our recent paper in the American Journal of Respiratory and Critical Care Medicine [8], we demonstrated high expression of several FGF/FGFR family members and overexpression of FGFR1, FGF2 and FGF18 in MPM cell lines and tissues as compared to normal mesothelial cell lines and the pleura. Also, FGF1, FGF5, FGF7 and FGF9 showed high expression in subsets of MPM cell lines. Altogether, this suggests an important role of FGF signals in MPM. Figure 1A shows immunofluorescent staining of FGFR1 in normal mesothelial cells, and in cell lines established from epithelioid and biphasic MPM. The upregulation of FGFR1 and FGFs appears to occur via epigenetic mechanisms since neither our own data nor the investigations by Marek *et al* [9], Plönes *et al* [10] or Shukuya *et al* [11] provided evidence of mutations or gene amplification in the FGF/FGFR family in MPM. This is in contrast to other thoracic malignancies where amplification of FGFR1 was found in about 20% in squamous cell carcinoma of the lung [12], 5% in small cell lung cancer [13] and 9% in esophageal cancer [14].

MPM has a very poor prognosis with an overall survival between 4 and 12 months due to the limited - mostly palliative - treatment options and to its aggressive growth and frequent resistance to chemo- and radiotherapy [15]. A major problem of MPM in the clinic is local recurrence after resection due to migration and invasion of tumor cells into the surrounding tissue. Current treatment options for MPM are either cisplatin/pemetrexed chemotherapy, radiation in late stage tumors, or trimodality therapy including induction chemotherapy followed by radical surgery and radiation [16].

Members of the FGF/FGFR family were shown to be potential therapy targets in various cancer types, including non-small cell lung cancer (NSCLC) [17], breast cancer [18], colorectal carcinoma [19], glioblastoma [20] and melanoma [21]. Regarding MPM, there have been earlier reports indicating a role of FGFs by showing high ligand expression in tumors and linking FGF2 with aggressiveness and poor prognosis [22-23]. We demonstrated that FGF-mediated signals indeed significantly contribute to the malignant phenotype of MPM. Treatment with recombinant FGF2 increased MPM cell proliferation and migration and activated ERK via the MAPK cascade. Ongoing experiments in our lab showed that 5 minutes of FGF2 treatment were enough to dramatically increase ERK phosphorylation, however, the phosphorylation was strongest after about one hour of ligand exposure an persisted for up to 24 hours (Figure 1B).

Blocking FGFR1 via the small molecule tyrosine kinase inhibitor (TKI) PD166866 or a dominant-negative FGFR1 adenoviral construct inhibited MPM cell proliferation, spheroid formation, migration, invasion and survival *in vitro* as well as xenograft tumor growth *in vivo*. Importantly, we also combined FGFR inhibition with cisplatin chemotherapy or radiation and found synergistic or additive activities. These data strongly point out the FGF axis as a potential therapeutic target in MPM and suggest that blocking FGFR-mediated survival signals could enhance the efficacy of other therapeutic modalities also in additional malignancies.

Several other RTKs were found to be overexpressed or hyperactivated in MPM, for instance EGFR, MET, and AXL. Their inhibition - especially in combination settings - showed promising effects in preclinical studies [24-27]. However, in clinical trials, erlotinib [28] and gefitinib [29] (two TKIs targeting EGFR) had only modest effects. Also antiangiogenic treatment with the pan-VEGFR inhibitor cediranib [30] or with the multikinase inhibitor sorafenib [31] (blocking predominantly VEGFRs and PDGFRs) showed only limited activity.

In summary, our study demonstrated that several FGFs and their receptors are highly expressed in MPM and their signals are major contributors to malignant cell behavior. Blocking these signals dramatically reduced tumor growth, enhanced the activity of other therapies and may have the advantage of also blocking MPM-induced angiogenesis. A schematic representation of the role of FGF signals in MPM is depicted in Figure 1C. Our data provide strong evidence for considering FGFR inhibition in combination with chemo- or radiotherapy as a new therapeutic approach in MPM. Translation of FGFR inhibition into systemic MPM therapy is currently evaluated in a recently launched phase IB trial (Trial ID: NCT01868022) using the FGFR ligand trap FP-1039 (GSK3052230) in combination with first-line chemotherapy as well as in a phase II trial (Trial ID: NCT01907100) using the multikinase inhibitor nintedanib targeting VEGFR, FGFR and PDGFR.

**Conflicting interests**

The authors have declared that no competing interests

exist.

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