bFGF/FGFR1 SIGNALING IS THE MAIN TARGET OF THE CYTOTOXIC ACTIVITY OF SORAFENIB IN HUMAN PLEURAL MESOTHELIOMA TUMOR-INITIATING CELLS

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Malignant pleural mesothelioma (MPM) is an aggressive and heterogeneous cancer which, after transient response to first-line chemotherapy, rapidly relapses leading to a dismal prognosis. MPM, as other solid tumors, contains a subset of self-renewing tumor initiating cells (TICs) that contribute to tumor formation, recurrence, metastasization, and drug resistance. Thus the characterization of TIC-peculiar survival and proliferative mechanisms is essential to the identification of drugs targeting these cells and improve therapeutic response. Pre-clinical studies reported significant results in MPM using tyrosine kinase (TK) inhibitors, including the multi-kinase inhibitor sorafenib (SOR), however most of them failed to achieve the expected survival benefits when translated into the clinics.

The aim of this study was to investigate the effects of SOR on fully-characterized TIC cultures (named MM1, MM3 and MM4) isolated from human MPMs and the intracellular mechanisms involved.

Cell viability/proliferation was investigated by MTT assay and BrdU incorporation, cell cycle and apoptosis analyses were carried out by FACS. Western blotting was performed to detect the modulation of protein expression and the phosphorylation status of intracellular mediators.

SOR significantly decreased viability and DNA synthesis of all the three cultures in a concentration- and time-dependent manner, and inhibited G1/S transition accumulating cells in the G0/G1 phase. These effects were associated with induction of apoptosis and downregulation of the anti-apoptotic factor Mcl-1.

Although SOR targets several TKs, its effects are usually ascribed to the direct inhibition of Raf kinase. To explore the intracellular effectors mediating anti-proliferative and pro-apoptotic effects of SOR, MPM TICs were stimulated with EGF to trigger MAPK activation. SOR caused a modest inhibition of MEK, ERK1/2, Akt and STAT3 after EGF exposure in all the cultures.

Since bFGF/FGFR1 signaling is relevant for MPM cell proliferation, we investigated the effects of SOR in TICs treated with bFGF. We observed a significant increase in MEK and ERK1/2 activation in MM3 and MM4,. SOR completely abolished bFGF-dependent MEK, ERK1/2, Akt and STAT3 phosphorylation, and the powerful activation of FGFR1 upon bFGF stimulation in MM3 and MM4 cells, suggesting that FGFR1 could represent a primary target by which SOR acts in MPM TICs. In MM1, which secrete high levels of bFGF leading to autocrine activation of FGFR1 and constitutive phosphorylation of MEK-ERK1/2, the treatment with bFGF did not further increase their activity but we observed a higher sensitivity SOR, proposing FGFR1 as the main target of the drug. The pivotal role of FGFR1 in MPM TIC proliferation was confirmed by the dose-dependent cytotoxic activity of the FGFR1 inhibitor PD173074: MM1 cells showed higher sensitivity in agreement with

the high release of bFGF. PD173074 also prevented the activation of FGFR1 downstream MAPK signaling, reducing phospho-ERK1/2 levels in all the three cultures. Furthermore, to discriminate the relative contribution of either FGFR1 or Raf in SOR inhibition of MAPK pathway, we used the pan-Raf kinase inhibitor AZ628 which significantly suppressed the EGF-dependent activation of MEK and ERK1/2 in MM4 cells and, contrarily to SOR, also in MM1 cells.

Overall, our data demonstrated that SOR induces cytotoxic and pro-apoptotic effects in MPM TICs, mainly due to the direct inhibition of FGFR1 signaling, highlighting a novel Raf-independent mechanism. Moreover, autocrine bFGF production may represent a crucial factor for MPM TIC survival and proliferation. Accordingly, selection of patients with constitutive FGFR1 activation via autocrine loop may identify more responsive tumors to targeted drugs like SOR.

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