

#00025 Role of hypoxia in pemetrexed-resistance of mesothelioma mediated by proton-coupled folate transporter, and preclinical activity of new anti-LDH compounds

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Introduction

There are few effective therapies for malignant pleural mesothelioma (MPM), which remains one of the most lethal cancers. We previously demonstrated that low expression of the PCFT transporter, both at mRNA and protein levels, is associated with shorter survival of MPM patients treated with pemetrexed (Giovannetti et al., 2017). Since hypoxia has also been associated to antifolate-resistance (Raz et al, 2014), this study was aimed at elucidating key factors in pemetrexed resistance and hypoxia that may contribute to the rational development of novel therapeutic interventions against mesothelioma.

Methods

The levels of PCFT and of the hypoxia marker carbonic anhydrase-IX were determined by immunohistochemistry in tissue-microarrays of pemetrexed-treated patients. The contribution of PCFT expression and hypoxia on pemetrexed activity were evaluated in mesothelioma cells and spheroids, through siRNA-knockdown and PCR arrays. In vitro and in vivo studies on MPM cells and primary cultures from peritoneal mesothelioma (Deraco et al., 2010) evaluated the activity of novel lactate dehydrogenase-A (LDH-A) inhibitors (Granchi et al., 2011).

Results

The lowest PCFT levels were detected in tissues with the highest levels of the hypoxia marker carbonic anhydrase-IX. PCR-arrays of 84 genes (Hypoxia-RT2 ProfilerTM) in cells treated with PCFT-siRNA, spheroids and cells under hypoxic conditions demonstrated increased expression of LDH-A, which was successfully targeted by specific inhibitors both in vitro (especially when growing cells at O₂ tension of 0.1%), and in novel in vivo orthotopic models, characterized by hypoxic regions, as also detected with high-frequency ultrasound and photoacoustic imaging.

Discussion

Our findings suggest that low PCFT expression, associated with pemetrexed-resistance, could stimulate the increased expression of LDH-A as a feedback mechanism. Within the cells an increase in acidity caused by LDH-A activity could indeed result in greater extracellular acidification and a strong proton-guided force that would favor folate transport via PCFT, thereby linking folate transport with cell metabolism.

Conclusion

These results support a new therapeutic strategy targeting LDH-A in order to eradicate pemetrexed-resistant mesothelioma cells characterized by low-PCFT.

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