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GLUT1 AND LOX INHIBITORS AS PERSPECTIVE ANTICANCER AGENTS TACKLING GLUCOSE AVIDITY AND ECM REMODELING IN TUMORS

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Introduction

Most cancers have large hypoxic regions, which display an increase of the glycolytic metabolism leading to the production of lactate, providing cancer cells with adequate amounts of energy and anabolites. To this end, tumor cells generally overexpress glucose transporters (GLUTs), in particular GLUT1, which results in an increased uptake of glucose to support their less efficient energy production (Warburg effect). Therefore, therapeutic interventions aimed at reducing cancer glycolysis may be implemented by several strategies, including the development of inhibitors of glucose transporters. Furthermore, extracellular matrix (ECM) remodeling is one of the key processes preluding metastatic invasion, and it is promoted by several effectors, such as lysyl oxidase (LOX), an enzyme commonly involved in extracellular matrix maturation. LOX is up-regulated by HIF-1 and plays a critical role in the development of metastasis. Therefore, LOX inhibitors may represent an additional and innovative strategy for the treatment and the prevention of metastatic cancer.

Methods

We have developed various classes of compounds that are able to interfere with GLUTs (Granchi et al. 2015, Tuccinardi et al. 2013) and LOX (Granchi et al. 2009) by molecular design and chemical synthesis. Their effect on cell proliferation, apoptosis, migration and other key determinants of activity were evaluated by sulforhodamine-B and luciferase assays, FACS, wound-healing assay, and Quantitative PCR. The studies were performed in seven PDAC cells, including five primary-cell-cultures and 3D co-cultures with human stellate cells, in normoxic and hypoxic conditions.

Results

The IC_{50}s of the tested compounds ranged from 13.9 to 32.0 μM after 72-hour exposure. Notably, these compounds were still active in 3D co-cultures of these tumor cells with pancreatic stellate cells, which showed

increased resistance to gemcitabine and are more representative of the dense stromal compartment with core hypoxic areas of this tumor type, as detected by immunohistochemical stainings. Remarkably, one compound (PGL-14) showed a synergistic interaction with gemcitabine, increasing apoptosis induction and accumulation of ROS. Furthermore, the combination of these drugs reduced cell migration and enhanced in vitro sensitivity to anoikis, suggesting the ability of these compounds to inhibit metastasis.

Discussion

GLUT1 inhibitors were more active in hypoxia, but still active also in normoxia. Conversely, we did not detect cytotoxic effects using the LOX-inhibitors in normoxia (at concentration until 50 μM) since they were designed as bioreductively activated prodrugs, which are therefore activated only under hypoxic conditions. However, at O_2 tension of 1%, IC_{50}s were below 10 μM. As reported previously, LOX inhibition was associated with reduction of the mRNA levels of fibronectin, suggesting that it might also have impact on the interaction of tumor cells with the stroma that are mediated by integrins and fibronectin, regulating tissue stiffness (Coppola et al. 2017).

Conclusion

Interventions aimed at blocking the glycolytic activity or the extracellular matrix remodeling of tumors by means of newly designed molecules proved to exert a synergistic effect with clinically approved drugs, such as gemcitabine. These results seem to support the strategy of the simultaneous GLUT/LOX-inhibition in order to further sensitize hypoxic cancer portions to chemotherapy.

Bibliography

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