Poster

Extracellular vesicles secretion by Lenvatinib and Sorafenib in HepG2 cells and their effect on cell death and proliferation

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ABSTRACT

Motivation: Sorafenib, which acts on the RAF / MEK / ERK pathways through the inhibition of Raf kinase and different tyrosine kinases (VEGFR2, PDGFR, c-Kit receptors), is the drug currently used as a first-line treatment in hepatocellular carcinomas of advanced stage. It has recently been shown that Lenvatinib, another multi-kinase inhibitor, also improves mean progression-free survival and mean time to cancer progression. This finding motivated us to study the possible antiproliferative effects of Lenvatinib compared to Sorafenib, in addition to the secretion profile of extracellular vesicles in HepG2 cultures due to its recognized role in tumor progression and metastasis.

Methods: To determine the percentage of proliferating cells in culture, the incorporation of bromodeoxyuridine (BrdU) was used as a marker, while the analysis of the apoptotic activity was done through a colorimetric test that allows detecting the amount of caspase 3/7 existing in culture. It is well known that there is a connection between apoptosis and autophagy, so we decided to study the changes that occurred in the latter process after treatment. For this, the level of expression of LC3-II was determined through an SDS-PAGE coupled to a Western-Blot analysis. The changes produced in the expression of VEGFR-2 and EGFR were also monitored and, finally, the secretion profile of extracellular vesicles was studied through the analysis of the expression of different markers (Lamp1, E-Selectin, CD63, TSG101, Grp78, GM130, Annexin V and Prohibitin) in fractions enriched in exosomes, extracellular vesicles and apoptotic bodies.

Results and Conclusions: The results for the group treated with Sorafenib reproduced what has been described so far in the literature referring to hepatocellular carcinoma: decrease in cell proliferation caused by the downregulation of the expression of different growth factors (EGFR and VEGFR-2) and increase of cell death by apoptosis. However, Lenvatinib did not reproduce the pattern we expected for an antineoplastic drug, since it increased cell proliferation. With respect to the secretion profile of extracellular vesicles, no convincing results were obtained. We think that this could be due to the capacity of separation of the different fractions of the protocol used or to the difficulty of obtaining, from them, high amounts of proteins to proceed to its analysis by WB.

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