POTENTIAL OF NATURAL COMPOUND AS HEDGEHOG PATHWAY INHIBITOR FOR THE TREATMENT OF INTRAHEPATIC CHOLANGIOCARCINOMA

BACKGROUND AND AIM

Intrahepatic cholangiocarcinoma (**iCCA**) represents a rare cancer arising in the biliary tree, linked to an alarming fatality rate. It is subcategorized into *large* bile duct iCCA and *small* bile duct iCCA, according to the World Health Organization new classification. Regretfully, the high variability of iCCA at the molecular, genomic, histological and clinical levels makes these difficulties unmanageable. However, improvement in targeted therapy, surgical management, and molecular characterization have been made in the past few years. Indeed, the molecular pathogenesis of iCCA is intricate and involves multiple molecular networks: among them, **Hedgehog** (Hh) pathway plays a crucial role in many hallmarks of iCCA, such as tumor proliferation, survival, migration and epithelial-mesenchymal transition reprogramming. The main intent of this study is to prove the antitumor efficacy of a natural compound, named Glabrescione B (**GlaB**) inhibiting Hh pathway in experimental models of human iCCA, *in vitro*.

MATERIAL AND METHODS

Trypan Blue Exclusion test have been used to assess, at different time points, the dose-response of free GlaB and hyaluronic acid (HA)-encapsulated GlaB (to better convey the drug into the site of damage), an inhibitor of Gli1 (Hh downstream transcriptional factor). **Western blot** analyses have been used to evaluate the target protein level. **Wound-healing assay** has been established to evaluate the migratory activity of all cell lines subjected to the treatments. All experiments have been conducted in n.3 experimental replicates.

RESULTS

Our research shows a dose- and time-dependent reduction of cell proliferation by Trypan Blue Exclusion test in all cell lines both with free GlaB and HA-GlaB from lower to higher concentrations and from 24-hour to 96-hour incubation ($p<0.05$). Similarly, at the protein level, Gli1 knockdown, in a dose- and time-dependent manner, is demonstrated ($p<0.05$). Eventually, Wound healing assay preliminary data revelead a dose- and time-dependent decrease in wound edge reunification, leading to a lower migratory capacity. These data illustrate a better comprehension of a novel and putative way in the management of iCCA.

CONCLUSIONS

Hedgheog pathway dysregulation is known to be correlated with the development and progression of various cancers, including iCCA. The accomplishment of this study lays the groundwork for in vivo pre-clinical studies of HA-encapsuled GlaB in iCCA.