جامعة الإسكندرية
ALEXANDRIA
UNIVERSITY
Faculty of Science

Faculty of Science

Department of Biochemistry

Molecular transformation of tumorigenic stem cells (TSCs): A Novel Targeting Therapy for Cancer.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Biochemistry

In

Biochemistry

Presented by

Basma Adel Elsayed Abd Elaziz Elshaer

B.Sc. (Biochemistry/ Chemistry), Faculty of Science, Alexandria University, 2013

2018

Hepatocellular carcinoma is considered the sixth most common cancer in the world and the second leading cause of death after lung cancer. Cancer stem cells (CSCs) which are a small subpopulation in cancer have been proposed to be cancer-initiating cells, and have been shown to be responsible for chemotherapy resistance and cancer recurrence. The identification of CSC subpopulations inside a tumor presents a new understanding of cancer development because it implies that tumors can only be eradicated by targeting CSCs. Although advances in liver cancer detection and treatment have increased the possibility of curing the disease at early stages, unfortunately, most patients will relapse and succumb to their disease. Strategies aimed to efficiently targeting liver CSCs are becoming important for monitoring the progress of liver cancer therapy and for evaluating new therapeutic approaches. Herein, we provide a critical discussion of biological markers described in the literature regarding liver cancer stem cells and the potential of these markers to serve as therapeutic targets.

Current surface markers or a particular phenotype are used to identify CSCs. Several markers as CD133 and CD90 proposed in the literature to identify CSCs in liver cancer using cell surface antigens are enriched in LCSCs (isolated by FACS or Ab-conjugated magnetic beads). Additionally, cytokeratin 7 and 19 may also serve as relatively specific markers of LCSCs and play significant roles in hepatocellular carcinoma. Also there are various transcription factors and signalling pathways specific to target LCSCs, as Aldehyde dehydrogenase, Notch, β-catenin, ERK1/2, Nanog, OCT-4, Cyclin-D1 and SOX2.

The present study focuses on LCSCs isolation specially $CD133^+$ /$CD90^+$ LCSCs from human tumor specimens and hepatic cell lines. Determination of the anti-cancer efficiency of Piperlongumine (PL) as a novel targeting therapy for $CD133^+$ /$CD90^+$ LCSCs. Examination the effect of PL on $CD133^+$ /$CD90^+$ LCSCs morphology, caspase activity, apoptotic induction, and their ability to inhibit colonies formation. Identify the effect of PL on ALDH6A1, Notch-1, β-catenin, ERK1/2 and CyclinD1 as main markers of LCSCs. Investigate the effect of PL on ALDH6A1 nuclear and cytoplasmic localization.

Piperlongumine:(5,6-Dihydro-1-(1-oxo-3-[3,4,5-trimethoxyphenyl]-trans-2-propenyl)-2[1H]-pyridinone), which is isolated from *Piper longum*, is a cell-permeable inducer of cell-death in cancer cells. It has been shown to inhibit expression of Bcl-2 and enhance wild type p^{53} expression. Piperlongumine is an inhibitor of GSTP1. PL has been traditionally utilized for treating respiratory and gastrointestinal disorders in an alternative medicine. PL has been demonstrated to have anti-angiogenic, anti-diabetic and anti-bacterial activities. Additionally, it has been found to have variable pharmacological effects and activities, as anti-aggregation of platelets, antifungal and analgesia features. In hyperlipidemic rats, PL has a cytotoxic effect on cancerous cells, also can promote the regulation of blood lipids metabolism. The PL stimulated selectively the accumulation of ROS in cancer cells represents a novel therapeutic strategy for cancers. Newly, PL was shown to have anti-tumorenic activity; it stimulates high levels of reactive oxygen species (ROS), resulting in cell death *in vitro* and *in vivo* by activating various mechanisms such as p38/JNK, MAPKs-CHOP, NF-kB pathways.

ALDHs have been shown in the normal liver tissue as a marker of liver progenitor cells and also in HCC as a CSCs marker. Thus, in HCC the ALDH expression is believed to be slightly different from ALDH bright cells. Besides that, these findings indicate that the ALDH expression when increased associated with an indicative factor of a well-morphological, differentiation and compatible prognosis in HCC.

An evolutionarily conserved pathway Notch signaling pathway has been showed to promote the CSCs capability of self-renewal, proliferation, differentiation, angiogenesis, and migration in several malignancies. Also, it controls abundant developmental processes, including the determination of cell fate, terminal differentiation and cell proliferation. There are several Notch inhibitors has been reported to be developed, as Notch is one of the most vigorously studied which act as CSCs therapeutic targets. It has been reported that $CD90^{+}$ cells which was isolated from normal and cirrhotic livers, also from HCC patients' tumor tissues, and blood samples expressed a high level of Notch1 compared with $CD90^{-}$ cells. It has been found that Notch1 has the ability to regulate the arteriovenous differentiation and in the hepatic endothelium of adult mice. Also, it is required in hepatic sinusoids for vascular homeostasis by

enhancing quiescence and differentiation of liver sinusoidal endothelial cells. So, Notch1 pathway disruption results in intussusceptive angiogenesis and nodular regenerative hyperplasia.

In HCC, there are several signaling pathways that have been found to desregulate such as PI3K/AKT/mTOR, RAS/RAF/MAPK, IGF, VEGF, HGF/c-MET, PDGF and WNT/β-catenin pathways. Among them, the most difficult to treat is the desregulation of WNT/β-catenin pathways. It has been observed that aberrant activation of the WNT/β-catenin pathway found in relatively 1/3 of HCCs. In LCSCs, accumulation of nuclear and cellular β-catenin is a hallmark of canonical WNT/FZ signaling activation that observed in 33–67% of tumors. Approximate 20% of HCCs have β-catenin gene mutations.

Results:

In our study, we successfully isolated $CD133^+/CD90^+$LCSCs from both primary human tumor specimens and hepatic cell lines. Then the anti-cancer effect of Piperlongumine treatment was investigated on cell viability (WST-1 assay) and proliferation to target LCSCs, which has a significant inhibition on the growth of LCSCs after treatment with different concentrations for 48hrs. We found that the PL at a concentration of IC_{50} value decreased cell viability of isolated $CD133^+/CD90^+$LCSCs by 51% after 48 hrs.

In addition, treatment of isolated $CD133^+/CD90^+$ LCSCs with PL showed dramatic morphological changes after 48 hrs. Also, it was found that PL induces apoptosis by using Enzyme Linked Immunosorbent Apoptotic Assay (ELISA) and also through determination of caspase-3 activity. The effect of tested compound PL showed reduction in the number of colonies formation in a dose dependent manner through colonogenic assay.

Targeting the main pathways and transcription factors in LCSCs, which are involved in the existence, recurrence, and relapse of the tumor. We found that PL was targeting isolated $CD133^+/CD90^+$LCSCs through inhibition of the protein expression levels of Aldehyde dehydrogenase (ALDH6A1), Notch-1, β-catenin, ERK1/2, and Cyclin-D1 using western blot analysis after 48hrs treatment. Furthermore, the anti-cancer efficiency of PL was examined by

immunocytochemistry (ICC) to determine the localization and expression of Aldehyde dehydrogenase (ALDH6A1).

Conclusion:

Piperlongumine (PL) inhibited the survival, proliferation, colonies formation, and stemness of $CD133^+/CD90^+$ LCSCs. Additionally; it enhanced caspase-3 activity and apoptotic induction of $CD133^+/CD90^+$ LCSCs. At the protein level, our data showed that PL decreased the protein expression levels of Aldehyde dehydrogenase (ALDH6A1), Notch-1, β-catenin, ERK1/2, and Cyclin-D1 after 48h of treatment compared to control. Also, treatment with PL showed reduction in the nuclear and cytoplasmic Aldehyde dehydrogenase (ALDH6A1) intensity of $CD133^+/CD90^+$ LCSCs. PL has an important role in targeting and eradicating the $CD133^+/CD90^+$ LCSCs.