Evaluations of Hexokinase-2 Inhibition with Liposomal Formulations of 3-Bromopyruvate using 3D Spheroid Models of Tumor Aerobic Glycolysis

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ABSTRACT

Purpose: To evaluate expression levels of glycolytic markers of tumor aerobic glycolysis using 3D cellular spherical models of human ovarian adenocarcinoma (SKOV3) and to investigate whether targeted liposomal formulations encapsulating 3-bromopyruvate (3-BPA), an inhibitor of glycolysis, can be used for more effective delivery and therapy.

Experimental Methods: SKOV3 cellular spheroids were grown in culture for 3 and 5 days using the hanging drop method. Hexokinase activity was determined by enzymatic assay in both 2D culture (under normoxia and hypoxia) and 3D spheroids. MTT assay was performed to determine the IC_{50} value of 3-BPA under hypoxia and normoxia. A reverse-phase HPLC analysis method was developed for the sensitive detection of 3-BPA. PEG-modified 3-BPA-loaded liposomes were formulated by thin film hydration technique for 3-BPA encapsulation and its delivery.

Results: SKOV3 cellular spheroids were roughly circular in shape and 200-400 μm in diameter. Hexokinase assay results confirmed higher HK activity levels in 3D spheroids and hypoxic (0.5% O_{2}) conditions compared to 2D SKOV3 cell cultures grown under normoxic (21% O_{2}) conditions for 5 hours. The hydrodynamic diameter of the spheroid was around 160 μm and a surface charge around +17 mV. Higher IC_{50} values were observed for 3-BPA SKOV3 cells grown under hypoxic conditions when compared to normoxic conditions. MTT assay showed that cells grown under hypoxic conditions gave a higher value. HK inhibition studies showed that the liposomal formulation potency was potent at later time points such as 12 hours and 24 hours.

Conclusions: The preliminary studies showed that cell grown under hypoxic conditions (both spheroids and hypoxia) lead to elevated hexokinase levels and lead to drug resistance indicating that biological relevance of spheroids to perform in vitro studies. Also, encapsulation of 3-BPA in PEG-modified liposomes leads to enhancement in activity of the therapeutic agent.

Ovarian Cancer: Ovarian cancer is the leading cause of death among gynecological cancers with an estimated 14,270 fatalities in 2014 in the United States (1). The main reason for such a high number of deaths is because majority of the cases (60%) of ovarian cancer are diagnosed at advanced stages, for which the 5 year survival rate is only 27% (2).

Tumor Aerobic Glycolysis – Causes and Consequences:

- Hypoxia
-Mismatched signals
- Metabolic defects

3D Tumor Cell Spheroids as Models of Aerobic Glycolysis:

Spheroids are micro-scale cell clusters which are formed by self-assembly of the culturing cells in 3-D and closely mimic in vivo physiological conditions such as hypoxia–cell contact geometry, chemical gradients and mass transport. Since hypoxia is known to up-regulate the expression levels of both the glycolytic mRNAs and protein levels of the hypoxic subpopulation and this metabolic alteration turns out to be very critical for testing the anti-cancer therapeutics because the efficacy of the drugs which target the glycolytic enzymes is altered in spheroids and these efficacy results give a more accurate picture when the same drug is tested in vivo.

RESULTS

Development of SKOV3 Spheroids

- Dispersase Cell Scapsulation
- Hanging Drop

| Dispersase | Hanging Drop | Aggregate | Spheroid |

| 96 well hanging drop plate |

| Microscopic images of SKOV3-spheroids |

CONCLUSIONS

- SKOV3 spheroids were successfully developed for evaluating hexokinase(HK) as a tumor aerobic glycolysis marker.HK activity levels were found to be elevated in cells grown under hypoxic conditions (both spheroids and hypoxia) on day 5 when compared to normoxic conditions.
- Evaluation of cytotoxic activity of 3-BPA under hypoxic and normoxic conditions revealed higher IC_{50} values for cells grown under hypoxic conditions indicating drug resistance.
- A reverse phase HPLC method was developed for the detection of 3-BPA and the lowest concentration that could be detected was 10 μg/ml.
- 3-BPA was encapsulated in to the hydrophilic core of the liposomes and the encapsulation efficiency was found to around 55%. 3-BPA loaded liposomes were characterized for size, surface charge and morphology by using TEM.
- HK inhibition study showed that liposomal formulations of 3-BPA were found to be more potent than solution form of 3-BPA at 12 hour and 24 hour time points.

References: