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http://dx.doi.org/10.5339/qfarc.2016.HBPP3364

Anti-Neoplastic Effects of Annonacin against Renal Cell Carcinoma

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Background and Objectives

Renal cell carcinoma is the most common and lethal form of all renal cancers, and accounts for 4.1% of all cancer cases in Qatar. Mutations to Von-Hippel Lindeau (VHL) gene in renal cells activates hypoxia inducible factor-1 alpha (HIF-1α) response pathway, and contributes to increased proliferation and progression to renal cell carcinoma. Hence, chemotherapeutic modalities available to treat renal cell carcinoma are targeted toward modulation of the VHL-HIF response pathway. Annonacin, a potent cytotoxic mono-tetrahydrofuran acetogenin found in Annonaceae plants, has been demonstrated to exert anticancer activity against breast cancer; however, its therapeutic potential against renal cell carcinoma is yet to be determined. Hence the objective of this study is to investigate anti-neoplastic potential of annonacin in renal carcinoma cells.

Methods

We investigated the effect of annonacin - at concentrations ranging from 0.5 to 2 µM - on cell viability (using MTT assay and Alamar blue assay), and the protein expression of markers of HIF signaling pathway (HIF-1α), mTOR pathway (Thr-389 phosphorylation of p70S6 kinase), cell cycle progression (p21 levels), and apoptosis (caspase-3 expression) in CaKi-2 cells, a human renal carcinoma cell line. The cells were treated with annonacin for 24 or 48 hours and assessed for the aforementioned parameters.

Results

48 hour annonacin treatment caused a significant and dose-dependent decrease in the viability of CaKi-2 cells, i.e., 42% in 0.5 µM, 36% in 1 µM and 29% in 2 µM annonacin treatment groups as compared to control set at 100%. This

Cite this article as: Munusamy S, Gopalakrishnan A, Ravindran S, Alali F, Eid AH. (2016). Anti-Neoplastic Effects of Annonacin against Renal Cell Carcinoma. Qatar Foundation Annual Research Conference Proceedings 2016: HBPP3364 http://dx.doi.org/10.5339/qfarc.2016.HBPP3364.

was further confirmed by Alamar blue assay, which revealed a significant decrease in the viability of CaKi-2 cells upon treatment with annonacin for 48 h. The expression of HIF-1α was reduced by 68% at 24 h in CaKi-2 cells treated with 2 μM annonacin. In addition, the expression of p21 (a key molecule that inhibits transition of cells from G1 to S phase in cell cycle) was induced by 1.34-fold in 0.5 μM annonacin-treated cells indicating an arrest in G1 phase of cell cycle. This was further confirmed through cell cycle analysis using Tali cytometer, in which annonacin treated groups (0.5 μM and 1 μM) showed cell cycle arrest at G1 phase, i.e., 57% of cells in G1 phase with 0.5 μM annonacin treated vs. 7% of cells in G1 phase in control group. In addition, a dose-dependent decrease in the phosphorylation of p70S6 kinase (a downstream target of mTOR) was observed with annonacin treatment at both 24 and 48 h end-points. This suggests that treatment of annonacin has possibly led to the inhibition of mTOR, in addition to suppression of HIF-1α activation, and underscores the cross-talk between HIF pathway and mTOR signaling pathway in renal cell carcinoma.

Conclusions

Our findings demonstrate that annonacin treatment (at concentrations ranging from 0.5 to 2 μM) inhibits HIF-1α and mTOR activation and causes cell cycle arrest at G1 phase and induces apoptosis in renal cell carcinoma. These findings indicate that annonacin exerts anti-cancer effects via modulation of HIF and mTOR signaling pathways, resulting in alterations in the cell cycle and activation of apoptosis in renal cell carcinoma. In conclusion, our study for the first time unveils the therapeutic potential of annonacin to inhibit the progression of renal cell carcinoma. Further studies in vivo are required to establish its efficacy to treat patients with renal cell carcinoma.

Funding Source

This study is supported by an intramural grant (#QUUG-CPH-CPH-14/15–7) funded by the Office of Academic Research, Qatar University, Doha, Qatar.