Lactoferrin-Doxorubicin conjugates as platform for improved cellular delivery of Doxorubicin to target hypoxia
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Highly aggressive and rapidly growing tumors have hypoxic (low oxygen) regions that can activate several signal transduction pathways to induce tumor angiogenesis and progression. Most of the mechanisms involved in hypoxia are due to the up-regulation of hypoxia inducible factor - 1α (HIF-1α). HIF-1α is a key regulator for the induction of several genes that are responsible for cellular adaption and survival at hypoxia. They are the potent inducer of angiogenic factors like vascular endothelial growth factor (VEGF) and erythropoietin and major stem cell marker like CD133 and multi drug resistance marker P-glycoprotein (P-gp). Hence there has been a growing interest to study the biological role of HIF-1α to overcome the associated poor prognosis. The present investigation describes the use of biologically active bovine milk glycoprotein lactoferrin (Lf) for targeting hypoxia. Lf has been proved to be a potent anti-cancer agent with several immuno-modulatory functions [1, 2]. Iron saturated bovine-Lf promisingly decreased the load of HIF-1α, anti-apoptotic proteins expressed in most cancers and VEGF as confirmed using flow cytometry and western blotting. Cancer cells were able to readily internalise Lf via Lf-receptors through receptor mediated endocytosis and decreased P-gp activity within the cells. This promising result led to the synthesis of bLf conjugates to improve cellular delivery of most widely used chemotherapeutic drug known as doxorubicin (Dox). The synthesized bLf-Dox conjugates were able to internalise into cells at both normoxic and hypoxic conditions. Interestingly, significant reduction (p ≤ 0.01) in P-gp expression was also noticed in cells treated with both bLf and bLf-Dox conjugates. Cells pre-treated with iron saturated bLf-Dox conjugate did not form spheroids when cultured until 72h. Caspase-3 expression was also found to be up-regulated with bLf-Dox conjugate treatments demonstrating the activation of apoptosis mediated cell death. The action of bLf-Dox conjugates on poly giant cancer cells (PGCC) which represents resistant form of tumor cells [3] were also studied using 400 μM CoCl2. bLf-Dox conjugates reduced the occurrence of PGCCs in the culture when compared to Dox alone treatments which showed increased number of PGCCs. Western blotting analysis proved the down-regulation of HIF-1α and CD133 in PGCCs treated with bLf-Dox conjugates. Generally, CD133 (+) cells are resistant to Dox alone treatments in solid tumors.

In our study, we found that bLf-Dox not only decreased the hypoxic load but also the stem cell marker CD133. Thus it can be envisaged that in future, bLf-Dox can be a potent drug conjugate to treat drug resistant tumors.

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