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Original article

Lifeguard inhibition of Fas-mediated apoptosis: A possible mechanism for explaining the cisplatin resistance of triple-negative breast cancer cells

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ABSTRACT

Triple-negative breast cancer does not express estrogen receptor-α, progesterone or the HER2 receptor making hormone or antibody therapy ineffective. Cisplatin may initiate p73-dependent apoptosis in p53 mutant cell lines through Fas trimerization and Caspase-8 activation and Bax up regulation and subsequent Caspase-9 activation. The triple-negative breast cancer, MDA-MB-231, overexpresses the protein Lifeguard, which inhibits Fas-mediated apoptosis by inhibiting Caspase-8 activation after Fas trimerization. The relationship between Fas, Lifeguard and cisplatin is investigated by down regulating Lifeguard via shRNA. Results demonstrate that cisplatin's efficacy increases when Lifeguard is down regulated. Lifeguard Knockdown MDA-MB-231 continue to decrease in cell viability from 24 to 48 h after cisplatin treatment while no additional decrease in viability is observed in the Wild-Type MDA over the same period. Higher Caspase-8 activity in the Lifeguard knockdown MDA after cisplatin administration could explain the significant decrease in cell viability from 24 to 48 h. This cell type is also more sensitive to Fas ligand-mediated reductions in cell viability, confirming Lifeguard's anti-apoptotic function through the Fas receptor. This research suggests that the efficacy of chemotherapy acting through the Fas pathway would increase if Lifeguard were not overexpressed to inhibit Fas-mediated apoptosis.

1. Introduction

About 10–20% of breast cancers are negative for estrogen, progesterone, and HER2 making hormone and antibody therapy ineffective. Cancers such as these are called triple-negative breast cancer [1]. Triple-negative breast cancer is often treated with surgery, chemotherapy or radiation therapy. A major drawback is that cancers such as triple-negative breast cancer can become resistant to certain chemotherapies [2]. Cisplatin has been shown to be a very effective single agent chemotherapy in recurrent or metastatic breast cancer [3]. Yet, cisplatin's efficacy is not without side effects. It is the most emetogenic of all chemotherapies and causes nausea, vomiting, hearing loss and general weakness in patients.

Cisplatin works by binding to DNA at sites of consecutive guanine bases and disrupts DNA replication and transcription at these binding sites, thus preventing cancer cells from proliferating [3]. In addition, treatment with cisplatin results in the transcription of proteins in the p53 family, including p73 [4], which leads to cell cycle arrest and the production of pro-apoptotic proteins such as Bax and Fas [2]. Bax promotes the production of Caspase-9 and cleavage of procaspase-3 with resulting apoptosis [5]. Cisplatin has also been shown to induce Fas-mediated apoptosis through Caspase-8 with subsequent cleavage of procaspase-3 and resulting apoptosis [6]. Unfortunately, cisplatin resistance is not uncommon and the mechanisms of resistance may be numerous and still not completely elucidated.

As mentioned, cisplatin extrinsically induces Fas-mediated apoptosis. However, MDA-MB-231 (a triple-negative breast cancer cell line) overexpresses an anti-apoptotic protein called Lifeguard (LFG) [7]. While the function and mechanism of LFG is not completely understood, researchers have found that LFG inhibits Fas-mediated apoptosis by binding to an allosteric site on the Fas

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receptor and inhibiting Caspase-8 activation that usually accompanies Fas receptor trimerization [8–11].

The purpose of this study was to investigate the relationship between Fas and Lifeguard to determine if LFG may function in affording resistance to Fas-mediated cell death and therefore chemotherapeutic resistance to cisplatin. To better understand the role of Lifeguard, protein production was down regulated using interference RNA (shRNA). Exogenous Fas Ligand was introduced to the wild-type MDA-MB-231 and Lifeguard knockdown (LFG Knockdown) MDA-MB-231 to reaffirm that Lifeguard inhibits Fas-mediated apoptosis. Furthermore, wild-type and LFG knockdown cells were introduced to varying concentrations of cisplatin to determine the cytotoxicity of the drug and to determine differences in cell viability between the cell lines in an effort to elucidate the relationship between cisplatin and LFG in Fas-mediated apoptosis. Dermal fibroblasts were used as a non-malignant cell control to examine side effects of cisplatin.

2. Materials and methods

2.1. Cell culture

MDA-MB-231 (MDA) cells were obtained from American Type Culture Collection (ATCC, Manassas, VA, #HTB-26). This cell line was chosen for the study as it was the only triple-negative breast cancer previously identified to overexpress LFG [7]. The complete growth medium consisted of DMEM/F12 (Life Technologies, Grand Island, NY, #10565018), 10%FBS (Life Technologies, #10438018), and 1%Penn/Strep (Life Technologies, #15140122). In all cases, the cells were incubated at 37 °C at 5% CO_2 with 100% humidity in

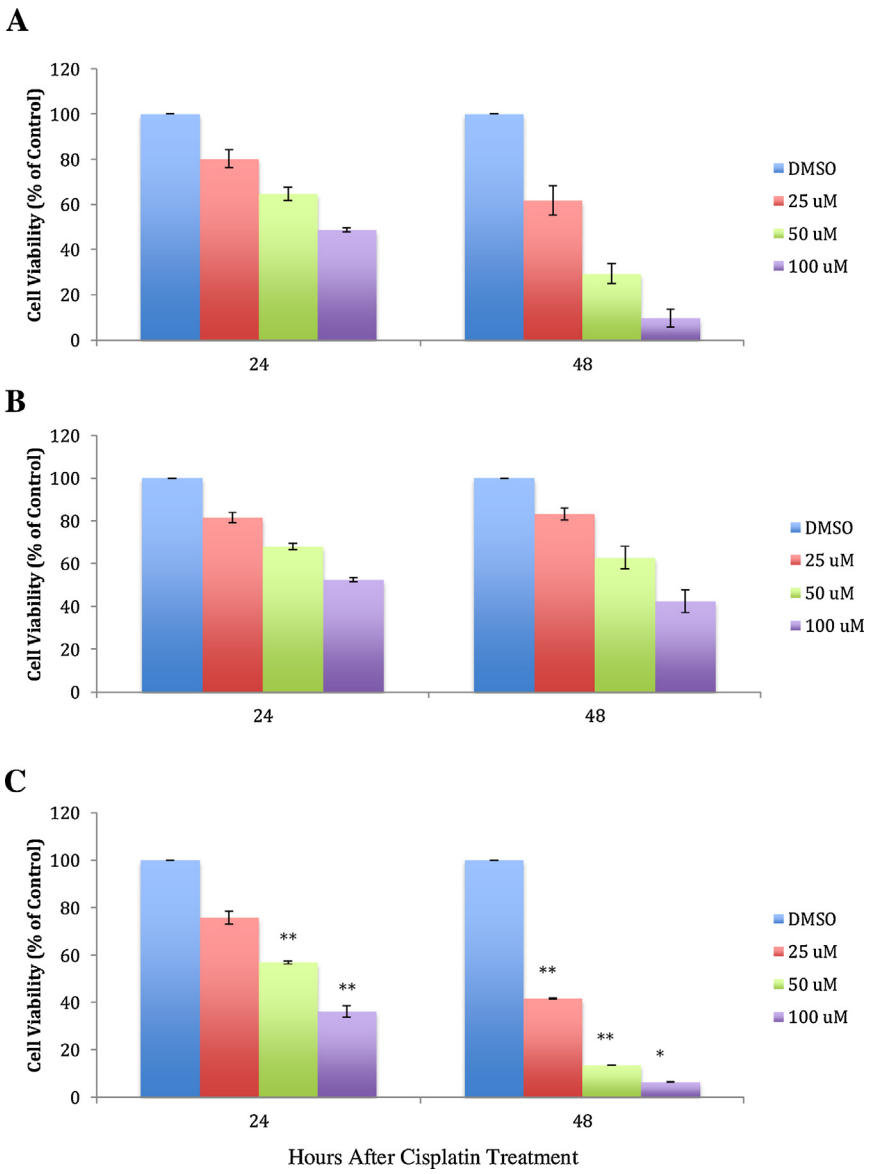

Fig. 1. Cisplatin sensitivity is dependent upon Lifeguard production.
Cisplatin cytotoxicity on non-malignant cells was examined using dermal fibroblasts. Cisplatin induces cell death in a dose-response and time-dependent manner. Results from two separate experiments are shown as mean ± SEM ($n = 4$). $p < 0.01$, ANCOVA comparing 24 and 48 h data.
Cisplatin's oncolytic activity was shown on wild-type (GFP+) MDA-MB-231. Cisplatin solely operates in a dose-dependent response where Lifeguard is over expressed. Results from three separate experiments are shown as mean ± SEM ($n = 5$). $p = 0.15$, ANCOVA comparing 24 and 48 h data.
In Lifeguard knockdown MDA, cisplatin administration produces a time-dependent and dose-dependent decline in cell viability. Bars are means ± SEM ($n = 5$). ($p < 1.0E-5$) ANCOVA, comparing data at 24 and 48 h. This data is representative of three to five independent experiments. *$p < 0.05$, **$p < 0.01$, t-test, when compared to wild-type MDA data of the same time period and cisplatin concentration.