Alexandria University
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A Ruthenium Compound as a New Drug for Breast Cancer Treatment

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ABSTRACT

Breast cancer is the most frequent malignancy with high morbidity and mortality among women worldwide accounting for 25% of female cancers worldwide. Mortality in breast cancer patients (14.7% of female cancer deaths worldwide) is mostly caused by metastasis which is related to poor prognosis of breast cancer patients. In Egypt, breast cancer is estimated to be the most common cancer among females accounting for 38.8% of all female cancers. In breast cancer, a complex molecular interplay involving multiple signaling pathways, including STAT3 signaling, and S6 kinase1 signaling have been attributed to the development of this disease. In addition, we tested the expression level of LC3B protein as a hallmark of autophagy induction.

We examined the effect of $Ru(quin)_2$ on ER+ human breast cancer cell line, T-47D, proliferation, apoptosis and autophagy induction. We found that $Ru(quin)_2$ inhibited cell viability and induced apoptosis and autophagy at IC_{50} (50 µM) for T-47D cell line. Morphological examination of T-47D cell line revealed promising anti-proliferative effects of $Ru(quin)_2$ in dose dependent manner. Our data suggested that the morphological changes of cell line started after treatment with 1/10 and 1/2 IC_{50} of $Ru(quin)_2$. Almost all treated cells showed cytoplasmic condensation, shrinkage, tendency to float in the medium, and reduction in size in comparison to the control after treatment with $Ru(quin)_2$ for 24h. Additionally, histone release was detected by ELISA which showed a high percentage of apoptotic induction for treated cells of T-47D breast cancer cell line. Furthermore, our data indicated that the T-47D showed a significant increase in caspase-3 activity in treated T-47D cells if compared with untreated cells after 24h, thus the sensitivity of the T-47D cell line to $Ru(quin)_2$ induced cell death was verified. Besides, cell cycle analysis was performed to examine and confirm the effect of $Ru(quin)_2$ treatment on apoptosis induction. The present data revealed that 1/10 IC_{50} and 1/2 IC_{50} of $Ru(quin)_2$ resulted in G2/M phase cell cycle arrest with apoptosis induction in T-47D for 24h.

It was found that $Ru(quin)_2$ reduced STAT3, S6 kinase1 expression patterns and enhanced LC3B expression levels in T-47D cell line, suggesting a novel mechanism of autophagy induction by $Ru(quin)_2$. We examined the effect of $Ru(quin)_2$ on the expression of LC3B on T-47D cell line, using immunocytochemical analysis, which showed that in the control cell, localization of LC3B was strongly expressed in the nucleus of T-47D cell line. When the cells were treated with 1/2 IC_{50} and IC_{50} of $Ru(quin)_2$ for 24h, the expression of LC3B protein level was significantly increased in T-47D cell line, and LC3B was transferred from nuclei to cytoplasm.

The present study introduced $Ru(quin)_2$ as a promising anticancer agent with therapeutic potential to induce autophagy through LC3B upregulation and inhibition of the downstream signal S6 kinase1. In addition, the anti-cancer effect of $Ru(quin)_2$ that resulted in apoptosis induction through caspase-3 activation and histone release, this finding may attribute to the downregulation of STAT3. Furthermore, flow cytometric analysis results confirmed the apoptotic effect of $Ru(quin)_2$ treatment on T-47D with G2/M phase cell cycle arrest. The present study provides the first *in-vitro* observations of antitumor effects of a novel RuII-based compound, achieved by the induction of apoptosis and autophagy in human ER+ T-47D breast cancer cell line.