INTRODUCTION

One core hallmark of cancer cells is their ability to evade the immune system. (1,2) This lead to the emergence of immunotherapy as a remarkable mode of treatment that can be used solely or as an adjuvant to augment other traditional methods and help overcome their shortcomings (3).

Toll-like receptors (TLRs) are pattern recognition receptors and the FDA has approved TLR-7 agonist Imiquimod as an immunotherapeutic drug. The downstream effect of TLR activation is still an understudied field. (4) One possible outcome is autophagy. It is a cellular degradation system responsible for cellular homeostasis; it may also serve as a mediator of cell death. (5,6) TLR-signaling and autophagy interact in various complex mechanisms and further insight into these mechanisms and complexities could present enormous potential in paving the way for the success of immunotherapy. (7,8)

METHODOLOGY

This study was conducted at (CERRMA), Faculty of Medicine, Alexandria University.

1- Immunofluorescence Staining

SCC4 cells were treated with Imiquimod (United States Pharmacopeia (1338313 USP)). Briefly, unconjugated primary polyclonal antibody TLR-7 (Rabbit, anti-human)(Abcam, ab45371) was added. The secondary antibody (Alexa fluor®488 goat anti-rabbit) was then added and then incubated with Hoechst stain 0.1-1 μg/ml. Examination was done by confocal laser scanning microscope (CLS); (Leica TSC SPE II/DMi 8).

2- Flow Cytometry

serves as a sensitive probe for flow cytometric analysis of the cells expressing autophagic vesicles.

SCC-4 cell line was divided into 3 groups. Group 1 was treated with 80 μg/ml of Imiquimod, group 2 with 2 μg/ml Cisplatin (Unistin, Hikma) for 6 hours, while group 3 received no treatment.

The fluorescent-activated cell sorter (FACS) flow cytometry assay assess the effect of Imiquimod on the induction of autophagy in oral SCC-4 cells using LC3B conjugated to Alexa Fluor®594 (R&D systems, IC9390T).

RESULTS AND DISCUSSION

1- Treatment of SCC-4 cell with Imiquimod leads to TLR-7 expression. (Fig. 1)

CONCLUSION

This study demonstrated that autophagy -responsible for stress-accommodation, as well as being a mediator of cell death- occurred downstream of TLR activation via Imiquimod. We also showed that Imiquimod surpassed traditional chemotherapy in inducing autophagy thus demonstrating a novel, off-label pattern in OSCC treatment. Further discernment of these mechanisms and pathways could greatly help in re-shaping the future of immunotherapy and cancer.

ACKNOWLEDGMENT

This work was supported by the Science and Technology Development Fund (30036).

REFERENCES