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IMIQUIMOD AND AUTOPHAGY; A NOVEL, OFF-LABEL PATTERN OF TREATMENT IN ORAL SQUAMOUS CELL CARCINOMA

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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 (ATCC® CRL1624™) 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 0.1-1µg. 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)

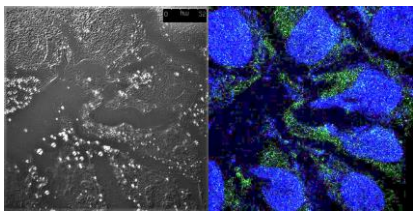

Figure (1): The fluorescent images of Imq-treated SCC-4 cells, labelled by TLR-7 Alexa fluor®488 reveal a bright green fluorescene in the cytoplasm. On the other hand, the nuclei exhibit the blue fluorescene of the hoechst counterstain.

2- TLR-7 agonist Imiquimod surpasses the gold standard stress inducer, Cisplatin in regards to autophagy-mediated cell death.

Our results reveal that in the untreated group, 4.1 % of the cells underwent autophagy while the cells treated with cisplatin resulted in 26.52 % autophagy. In the study group, Imiquimod resulted in 30.28 % of the cells expressing LC3B, fig. (2).

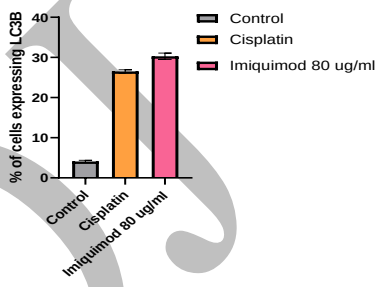

Figure (2): Using ordinary one-way ANOVA, the % of LC3B-stained cells was significantly increased in the Imiquimod-treated group compared to the control group. Imiquimod group expressed higher % of LC3B-expressing cells than the Cisplatin-treated group, but not significantly different.

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.

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REFERENCES

1. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Vol. 144, Cell. 2011. p. 646–74.
2. Hanahan D. Hallmarks of Cancer : New Dimensions. Am Assoc Cancer Res. 2022;(January):31–46.
3. Farkona S, Diamandis EP, Blasutig IM. Cancer immunotherapy: the beginning of the end of cancer? BMC Med. 2016 May 5;14:73.
4. Kaczanowska S, Joseph AM, Davila E. TLR agonists: our best frenemy in cancer immunotherapy. J Leukoc Biol. 2013 Jun 1;93(6):847–63.
5. Levine B, Klionsky DJ. Development by self-digestion: Molecular mechanisms and biological functions of autophagy. Vol. 6, Developmental Cell. 2004. p. 463–77.
6. Bhutia SK, Mukhopadhyay S, Sinha N, Das DN, Panda PK, Patra SK, et al. Autophagy: Cancer's Friend or Foe? Adv Cancer Res. 2013;118:61–95.
7. Jiang G-M, Tan Y, Wang H, Peng L, Chen H-T, Meng X-J, et al. The relationship between autophagy and the immune system and its applications for tumor immunotherapy. Mol Cancer. 2019;18(1):17.
8. Delgado MA, Deretic V. Toll-like receptors in control of immunological autophagy. Cell Death Differ. 2009;16(7):976–83.

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