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Investigating Role of Autophagy in Cispaltin Induced Stemness and Chemoresistance in Oral Squamous Cell Carcinoma

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Abstract : Background: Regardless of the development multimodal treatment strategies, oral squamous cell carcinoma (OSCC) is often associated with a high rate of recurrence, metastasis and chemo- and radio- resistance. The present study inspected the relevance of CD44, ABCB1 and ADAM17 expression as a putative stem cell compartment in oral squamous cell carcinoma (OSCC) and deciphered the role of autophagy in regulating the expression of aforementioned proteins, stemness and chemoresistance. Methods: A retrospective analysis of CD44, ABCB1 and ADAM17 expression with respect to the various clinicopathological factors of sixty OSCC patients were determined via immunohistochemistry. The correlation among CD44, ABCB1 and ADAM17 expression was established. Sphere formation assay, flow cytometry and fluorescence microscopy were conducted to elucidate the stemness and chemoresistance nature of established cisplatin-resistant oral cancer cells (FaDu). The pattern of expression of CD44, ABCB1 and ADAM17 in parental (FaDu-P) and resistant FaDu cells (FaDu-CDDP-R) were investigated through fluorescence microscopy. Western blot analysis of autophagy marker proteins was performed to compare the status of autophagy in parental and resistant FaDu cell. To investigate the role of autophagy in chemoresistance and stemness, sphere formation assay, immunofluorescence and Western blot analysis was performed post transfection with siATG14 and the level of expression of autophagic proteins, mitochondrial protein and stemness-associated proteins were analyzed. The statistical analysis was performed by GraphPad Prism 4.0 software. p-value was defined as follows: not significant (n.s.): $p > 0.05$;*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$; ****: $p \leq 0.0001$ were considered statistically significant. Results: In OSCC, high CD44, ABCB1 and ADAM17 expression were significantly correlated with higher tumor grades and poor differentiation. However, the expression of these proteins was not related to the age and sex of OSCC patients. Moreover, the expression of CD44, ABCB1 and ADAM17 were positively correlated with each other. In vitro and OSCC tissue double labeling experiment data showed that CD44+ cells were highly associated with ABCB1 and ADAM17 expression. Further, FaDu-CDDP-R cells showed higher sphere forming capacity along with increased fraction of the CD44+ population and β-catenin expression FaDu-CDDP-R cells also showed accelerated expression of CD44, ABCB1 and ADAM17. A comparatively higher autophagic flux was observed in FaDu-CDDP-R against FaDu-P cells. The expression of mitochondrial proteins was noticeably reduced in resistant cells as compared to parental cells indicating the occurrence of autophagy-mediated mitochondrial degradation in oral cancer. Moreover, inhibition of autophagy was coupled with the decreased formation of orospheres suggesting autophagy-mediated stemness in oral cancer. Blockade of autophagy was also found to induce the restoration of mitochondrial proteins in FaDu-CDDP-R cells indicating the involvement of mitophagy in chemoresistance. Furthermore, a reduced expression of CD44, ABCB1 and ADAM17 was also observed in ATG14 deficient cells FaDu-P and FaDu-CDDP-R cells. Conclusion: The CD44+/ABCB1+/ADAM17+ expression in OSCC might be associated with chemoresistance and a putative CSC compartment. Further, the present study highlights the contribution of mitophagy in chemoresistance and confirms the potential involvement of autophagic regulation in acquisition of stem-like characteristics in OSCC.

Keywords : ABCB1, ADAM17, autophagy, CD44, chemoresistance, mitophagy, OSCC, stemness
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