ADAR1, a promising "protecting" biomarker in oral squamous cell carcinoma

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AIM

Different studies evaluated the role of ADAR1 in different kinds of cancer, for which increasing of ADAR1 expression is linked to a worst prognosis and metastasis. Unclear results came from one study published about ADAR1 in oral squamous cell carcinoma (OSCC)[1]. Aim of our study was to perform a histologic analysis by immunohistochemistry in order to deeply evaluate the role of ADAR1 in OSCC patients.

MATERIALS AND METHODS

For the analysis of ADAR1 expression, we used a tissue microarray (TMA) including 54 patients, who filled informed consent. The source paraffin blocks were cored. A rabbit monoclonal antibody (Ab) supplied by abcam code [EPR7033] (ab126745), was used to perform the immuno-histochemical staining. Observational quantification analysis was performed by an experienced pathologist, according to two parameters: the intensity of staining and the percentage of the stained cells in both cytoplasm and nucleus. The intensity (I) of expression was scored from 0 to 3 (0 = no staining; 1 = yellow; 2=light brown; 3 = black brown/black). The proportion (%) of cells staining positively was scored from 1 to 6 (1 = 0-4%; 2 = 5-19%; 3 = 20-39%; 4 = 40-59%; 5 = 60-79%; 6 = 80-100%). Categorization of the scores in low (0-1/0-1-2-3) and high (2-3/4-5-6) intensity/percentage of positive expression for ADAR1, was undertaken in order to highlight a possible and different role of ADAR1 according to its allocation. SPSS 21.0 Software was used for the statistical analysis.

RESULTS

Multivariate Cox regression analysis explored how ADAR1 with different pattern of expression, in both nucleus or cytoplasm, could influence the patients' relapse and prognosis. Relapse-Free Survival (RFS) and Overall Survival (OS) were analyzed. Here were report only statically significant results. At Univariate analysis of Kaplan-Meier, lower percentage of stained nuclei, resulted to influence the recurrence status (p = 0,012). Lower number of nuclei stained resulted to be linked to an increase of appearance of relapses. We think this result should be analyzed farther in a bigger sample size, first of all, because in our cohort, there were not registered many relapse events. In the second place, Multivariate analysis failed to find this association. According to the OS, surprising results came from both Univariate and Multivariate analysis. ADAR1 resulted to be an independent prognostic factor when expressed in the cytoplasm. In particular from Kaplan-Meier analysis, the higher intensity of ADAR1 expression in the cytosol seems to be a favorable prognostic factor for the survival (p = 0,020). The higher number of stained cells in the cytoplasm, anyway, failed to find this association (p = 0,078). At multivariate analysis previous results were confirmed. ADAR1 resulted to be an independent protective prognostic factor in OSCC, when higher expressed (p = 0,003; Hazard Ratio 0,100; 95% C.I. 0,022-0,450). By this analysis, also Grade and Stage resulted to be independent prognostic factor, with a worst prognosis. Higher grade resulted to influence with an HR of 3,381, C.I. 1,116-10,239; p = 0,031. HR for Staging resulted to be 2,328; C.I. 1,157-4,684; p = 0,018.

DISCUSSION

It is reported that the most common type of cancer in men in south-central Asia is OSCC, with more than 200.000 new cases per year in the world. Even if treatment modalities got a great improve in the last years, 5-years survival rate for this disease, didn't improve as well, being below 50%. Thanks to an increase knowledge of tumor biology and technologies, we are able to better understand the events that could affect the normal cell in its change to a cancer cell. It's well known that accumulation of mutations it's an important mechanism, leading to a genetic change in the normal sequence of normal cell DNA. Anyway, these events are most in the cases supported by other mechanisms, which take the name of epigenetic and post-transcriptional events. In this view, one well-known epigenetic phenomenon is the RNA-editing. One of main character in this event is the ADAR1 enzyme,

which catalyzes a RNA change by modifying an adenosine to an inosine in the reading sequence. ADAR1, with two other isoforms, ADAR2 and ADAR2, belongs to the ADAR family. ADAR1 locus is on human chromosome 1q21.1-q21.2, made of 15 exons for a total of around 30 KBs. Its transcripts are generated and allocated in cytoplasm or nucleus. The reaction catalyzed is a deamination of adenosine to inosine, which is read as guanosine. Thus, the editing event is considered as an A-to-G "mutation" in the target RNA, with consequence in the transcription process and in the final protein structure. In many cancers ADAR1 resulted to be amplificated. In vitro experiments reported that upregulation of ADAR1 brought to an increase of cancerous characteristics in cells, such as proliferation and migration. The link between ADAR1 and this change in phenotype is still unclear, but the answer could be in the RNA-editing events that this enzyme could determinate. It is reported that in one case, in an experimental in vitro study of melanoma cells, ADAR1 resulted to be downregulated, but still linked to a malignant phenotype[2,3]. We firstly report, above all in OSCC, that ADAR1 increased expression in the cytoplasm is protective and influence the patients' prognosis. This result is against a previous study, which investigate the role of ADAR1 in OSCC patients. First, differences could be seen in the antibody used. That research team used a polyclonal antibody; meanwhile we choose a monoclonal one. This could explain the difference in binding the specific target, with a different signal in staining. They also did not perform a univariate or multivariate analysis on their sample. This could justify the difference in outcome. Also scoring system is substantially different: a differential expression pattern between nucleus of cytoplasm is not clear in that study. In conclusion, bigger sample sized study are needed, with a standardized scoring method. This would help in a better understanding of ADAR1 role in OSCC patients and prognosis. This clinical study should be integrated with bio-molecular studies. This kind of studies could help to clarify the role of ADAR1 in the different events that characterized cancer. What is sure, that ADAR1 could have different behavior according to the cancer type and above all its localization, according to which substrates are present in the cell. This relation, then, could influence the prognosis.

Keywords: biomarker, prognosis, RNA-editing, ADAR1, OSCC (Oral Squamous Cell Carcinoma)

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