Editorial

Potential application of human SALL4 on oral squamous cell carcinoma

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The study "*Study on the mechanism of SALL4 down-regulation in promoting the invasion and migration of oral squamous cell carcinoma and influencing the survival and prognosis of patients*" from Dr. Lin and collaborators, recently approved for publication at *Annals of Translational Medicine*, investigated the hole of human SALL4 in invasion and migration of Tca8113 cell lines, an oral squamous cell carcinoma (OSCC) cell line, originate from a female patient with tongue cancer. It was an exclusively experimental study, in which the authors evaluated the invasion and migration ability of miRNA transfected cell and the effects of SALL4 regulation related microRNA by transwell method. It was found that SALL4 expression was higher in upregulated group when compared to downregulated cells, without difference in SALL4 protein expression between both groups but with lower protein expression in control cells. Moreover, both groups presented lower rates of cell invasion and migration when compared to control cells, also demonstrating the targeted regulation of SALL4 by microRNA. The study's conclusion is that OSCC have low expression of SALL4 regulation-related microRNAs with low invasion and migration rates. If it is reflected in human beings, SALL4 could be a new treatment target to avoid local spread and metastatic disease.

Briefly, SALL4 belongs to the spalt-like gene family (SALL), in humans the gene 4 is located on chromosome 20q13.2. There are three SALL4 isoforms called SALL4A, SALL4B and SALL4C, with isoforms A and B being the most understood. The gene act in embryonic cells through the maintenance, self-renewal and pluripotency of embryonic stem cells (ESCs) and its action gradually decreases with the maintenance of organs and tissues (1,2).

SALL4 is widely expressed during murine embryonic development, in the adult organism the gene is silenced in most organs, but detectable in germ cells of testicles and ovaries, the same is believed to be found in adult humans and is also present in stem cells. In addition to expression in hematopoietic stem cells (HSCs), SALL4 is expressed in fetal hepatoblasts and silenced in adult hepatocytes (1,3).

In mouse, the heterozygous knockout causes organ dysplasia and the homozygous knockout causes embryonic lethality. In humans, the mutation of the *SALL4* gene causes Okihiro syndrome, an autosomal dominant disorder (2,4).

SALL4 is detected in 4-cell stage embryos and restricted to the cell mass from which the ESCs are derived. Isoforms A and B are components of formation of homedimers or heterodimers with DNA binding sites (5). *In vitro*, SALL4-translated HSCs expand rapidly, which is observed in

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contrast, through the depletion of endogenous SALL4, promoting proliferative reduction. In human bone marrow, SALL4 expression is only linked to $CD34^+$ HSCs and decreases after differentiation. In conclusion, the expression of *SALL4* gene regulates the maintenance and proliferation of HSC in short and long term (2,6).

SALL4 is physiologically expressed in embryonic development, described in the literature for being present in neoplastic diseases due to its re-expression. Its overexpression affects several cellular processes involved in the initiation of the neoplasia, growth, and tumor invasion. There are several mechanisms involved in this turn of expression, as well as the relationship between the *SALL4* gene and microRNAs (2).

The expression of SALL4 participates in the regulation of mechanisms of cell proliferation, apoptosis, migration and invasion and drug resistance, through several genes, according to a model proposed by Zhang *et al*. (2), contributing with tumor development and maintenance.

The return of SALL4 expression is related to the development of several neoplasms and its primary discovery was linked to germ cell tumors, correlating its function as a biomarker and molecular target for prognosis. Its expression was identified in, for example, lymphoblastic lymphoma, acute and chronic myeloid leukemia, and tumors of the digestive system, considering the latter of poor prognosis (2).

MicroRNAs are small non-coding RNAs that act as post-transcriptional regulators. There is a link between the regulation of microRNAs by the expression of SALL4. As consequence, they can promote mRNA degradation or translation repression of targets, through binding to the region 3'. Due to their ability to alter cellular processes such as cell proliferation, cell cycle, migration and apoptosis, microRNAs have become research targets and, as a result, their activity has been directly linked to several neoplasms acting as oncogenes and/or tumor suppressors (7,8).

Some studies identified the association of specific microRNAs to the oncogenic process, such as members of the Let-7 family that are found to act as tumor suppressors by inhibiting the c-Myc oncogene and miR-122, which suppresses, when negatively expressed, hepatocellular carcinoma via TGF-beta, for example (9).

Specifically in hepatocellular carcinoma, SALL4 re-expression was associated with decreasing survival. Moreover, levels of miR-98, a constituent microRNA of the Let-7 family, and SALL4 were inversely correlated in this tumor, suggesting that SALL4 expression can negatively regulate the expression of miR-98. In the same study, it was observed that the hyperexpression of miR-98 suppressed the expression of SALL4 in mouse and, consequently, the volume of the tumor (10).

The low expression of Let-7 microRNA family is considered also a predictor of poor prognosis in lung carcinomas, and tumors with EGFR mutation express more SALL4 when compared to normal tissue in addition to miR-98 being downregulated by the gene (11). In another study with lung carcinoma cell lines A549 and H1229, it was observed cell proliferation, migration, and invasion when SALL4 was overexpressed, also promoting downregulation of miR-98. In addition to the types of neoplasms mentioned, there is also a correlation between this microRNA and SALL4 expression in breast, ovarian and colorectal cancers (1,2).

A range of interactions of other different microRNAs with the re-expression of SALL4 were identified. The expression of miR-107, a microRNA related to several neoplasms and with tumor suppression or oncogenic function, in glioma cells is related to apoptosis and inhibition of tumor growth, suppressing the expression of SALL4 (12,13). Other examples are in gastric cancer that miR-16 has been shown to regulate the expression of SALL4 and in colon cancer by the expression of miR-219 (14,15).

The re-expression of SALL4 in cancer has several cellular processes in promoting the neoplasm. The regulation of cell proliferation is through the beta-catenin/cyclin D1, IMC and PTEN pathways. In the step involved in apoptosis, SALL4 negatively regulate HOXA9, FADD and BMI-1, which are promoters' regions of genes involved in apoptosis, and, consequently, blocks the process and the self-renewal of ons of cancer stem cells. This process is also regulated by Oct4, Nanog, Sox-2 pathways (16). It was also identified in endometrial carcinoma the binding of SALL4 to the c-Myc promoter region. This pathway could activate the neoplasm, while for lung cancer EGF was observed linked to the positive regulation of SALL4. In addition, SALL4 is not found in normal or hyperplastic tissue, which demonstrate that SALL4 expression is even more evident in several processes involved in tumorigenesis (17). In addition, high expression of SALL4 upregulates the expression of Twist1 and N-cadherin and downregulates E-cadherin, promoting the process of epithelial-mesenchymal transition (EMT), resulting in invasion and metastasis due to increased cell motility (2).

Squamous cell carcinoma represents the main histological subtype in HNC, with a major impact on public health, due to high morbidity and mortality rates, in addition to a low

survival rate (18). Tobacco use, alcohol abuse and human papilloma virus (HPV) infection are the main risk factors for HNC (18-20). OSCC is the sixth most common cancer globally, and recent studies have correlated the presence of cancer stem cells, one of the causes for the occurrence of this neoplasm (21,22).

Although there are several studies relating the aforementioned neoplasms to SALL4 expression, there is little information about its hole in head and neck cancer (HNC) (16). A study carried out by Ram *et al.* (23), in 2017, identified three populations of cancer steam cells in squamous cell carcinoma of the lip. Otherwise, the high expression of SALL4 was associated with low survival on OSCC (24). Kulkarni *et al.* (16), found that the expression of SALL4 was correlated with poorly differentiated OSCC, when compared to normal tissue, in addition to the correlation of the expression of SALL4 and cell proliferation. In another recent study, the expression of SALL4 mRNA in HNC samples was detected and its positive expression contributed to an increased risk of disease recurrence, in addition to greater aggressiveness. The authors further observed that its negative expression detected by siRNA in HNC cells caused a growth inhibition effect, also suggesting the hypothesis that SALL4 acts as an oncogene (25).

Despite the clinical importance of SALL4 expression and its correlation with several malignant tumors, its relationship on HNC remains unclear. The existing results so far in the literature support the hypothesis that SALL4 could be a potential biomarker for HNC and a potential target for drug development.

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