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EDITORIAL – GASTROINTESTINAL ONCOLOGY

Role of the Anoctamin Family in Various Carcinomas

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The anoctamin family (encoded by TMEM16 genes) includes 10 isoforms: ANO1–10 (TMEM16A, B, C, D, E, F, G, H, J, K).[1] Anoctamins have scramblase activity for phosphatidylserine, phosphatidylcholine, and galactosylceramide, in addition to channel activity.[2] Some recent experimental studies reported the correlation between anoctamins and cancer progression; however, only a limited number of clinical studies have been conducted, therefore additional studies are needed to clarify the roles of anoctamins in various carcinomas.

Among the anoctamin proteins, ANO1 (encoded by TMEM16A), which is located on chromosome 11q13, has been most precisely evaluated. This protein is also known as Discovered on GIST1 (DOG1), oral cancer overexpressed 2 (ORAOV2), tumor-amplified and overexpressed sequence 2, and FLJ10261.[3] ANO1 is expressed on the apical membrane of epithelial cells in airways and the gastrointestinal tract in humans, and is considered a molecular component of Ca^{2+}-activated Cl^- channels (CaCCs).[4,5] Moreover, changes in intracellular Ca^{2+} levels are linked to cell proliferation and migration, and can result in the development of certain cancers. Therefore, ANO1 is obviously associated with tumor cell proliferation and migration via CaCCs.[6] Some in vitro studies also found that ANO1 expression is correlated with cell proliferation, and RNA interference-induced ANO1 depletion or inhibition of CaCCs resulted in reduced cell proliferation.[7] Other studies found that signaling through epidermal growth factor receptors (EGFRs) and calmodulin-dependent kinase,[8] upregulation of insulin-like growth factor-binding protein 5,[9] and Cl^- transport were closely associated with ANO1 overexpression.[10]

However, other studies using GIST-derived ANO1-overexpressing cell lines reported contradictory results[9]; thus, the function of ANO1 may depend on the cancer cell environment.

Moreover, we must pay attention to reports indicating that ANO1 expression sometimes conversely affects normal cells.[11] Some experimental and clinical studies suggested that ANO1 amplification was closely associated with worse prognosis in squamous cell carcinoma,[12–14] while another study observed a correlation of ANO1 overexpression with advanced tumor stage in patients with non-small cell lung cancer.[15] Therefore, ANO1 was regarded as a prognostic factor for some cancers. As the link between cancer cell behavior and TMEM16 family members remains unclear, further clinical studies in different types of cancers are necessary to confirm the role of ANO1.

ANO6 (encoded by TMEM16F) exhibits calcium-dependent phospholipid scramblase activity, and CaCC currents are observed after overexpression of this anoctamin.[16] Similarly, as for ANO1, ANO6 is also activated during hypotonic cell swelling, and contributes to regulatory volume decreases under high intracellular Ca^{2+} concentrations. As a result, tumor cells migrate through the narrow gaps of endothelial cells after cell shrinkage. Moreover, ANO6 also contributes to decreasing the apoptotic volume.[17] These data suggest the important role of the anoctamin family in regulating tumor cell proliferation and apoptosis. Previous studies reported that ANO6 participates in cell migration, cell volume regulation, and apoptosis, thereby influencing cell growth and metastasis in human cancers. In particular, ANO6 RNA splicing influences the metastatic capacity of mammary cancers in mice, and is related with poor prognosis in breast cancer.[18]

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ANO9 plays important roles in physiological functions such as ion transport, phospholipid scrambling, and the regulation of other ion channels. A previous study evaluated the role and oncogenic mechanisms of colorectal cancer. The research found that ANO9 downregulation has an important role in the tumorigenesis and progression of colorectal cancer and concluded that greater understanding of the pathophysiological functions of ANO9 leads to improved outcomes through appropriate prognostication and molecularly targeted treatment.[19] The outcomes of this study differed from those of previous studies examining other anoctamins; no prior studies evaluated the correlation between ANO9 and colorectal cancer. Differences in cancer type, cancer stage, and number of patients can affect study outcomes. Further studies regarding the relationship of ANO9 with colorectal cancer and other cancers are mandatory.

Katsurahara et al. first evaluated the role of ANO9 in esophageal squamous cell carcinoma (ESCC) using experimental methods and clinical data.[20] It is noteworthy to focus on the correlation between ANO9 and ESCC. In an in vitro study, depletion of ANO9 resulted in reduced cell proliferation, invasion, and migration. Moreover, ANO9 depletion induced apoptosis. A microarray study revealed the involvement of centrosome-related genes in the regulation of ANO9. Clinically, patients with high ANO9 expression according to immunohistochemical analysis exhibited significantly worse survival. These findings clearly and reasonably demonstrated the role of ANO9 in ESCC. This comprehensive study is compatible with previous studies, and may thus direct future studies in the TMEM16 field. In particular, oncological studies of TMEM16, other than those involving TMEM16A, have been insufficient; thus, vigorous efforts are needed to establish the correlation between the TMEM16 family and various cancers.

The therapeutic outcomes of ESCC are not satisfactory after surgery or chemoradiotherapy; therefore, additional therapeutic strategies are urgently required to improve the therapeutic outcomes of ESCC. Some TMEM16 family members may initiate the process of tumorigenesis similar to oncogenes, or promote the progression of tumorigenesis. In either case, inhibition of some anoctamin functions may represent a potential treatment strategy for cancer, both alone and in combination with other strategies.

For example, we can anticipate that inhibition of STAT6, a novel agonist ANO1 promoter, decreases the proliferation, migration, and invasion of cancer cells. Luteolin, which also inhibits ANO1 channel activity and decreases protein expression levels of ANO1, can be a candidate of anticancer drugs. Furthermore, ANO9-induced EGFR upregulation is a predictive biomarker for adverse response to anti-EGFR therapy. Therefore, combination therapy with an ANO9 inhibitor may help prevent drug resistance and tumor relapse in several types of cancer. It is necessary to develop new anoctamin inhibitors for cancer therapy. For this goal, we need better comprehension of the structure–function relationship, physiological role, and regulation of each anoctamin using multiple approaches, including gene silencing in native cells, overexpression in null systems, and conditional knockout in mice.

As described previously, a few clinical studies argued that some TMEM16 family members function as adverse prognostic factors in a subset of cancers; however, these retrospective studies included small sample sizes, different patient characteristics, heterogeneous treatments, and a lack of multivariate analysis, making their reproducibility low. Therefore, it is important to conduct comprehensive studies according to the McShane Guidelines[21] to clarify the clinical role of the anoctamin family in different cancers.

In conclusion, TMEM16 could represent a useful diagnostic and prognostic marker for some cancers, and we can anticipate that it may be an important molecular drug target by analyzing the precise mechanism of its involvement in cell migration, cell volume regulation, and apoptosis in different cancers.

DISCLOSURE Chikara Kunisaki declares no conflicts of interest.

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