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Olfactomedin 4, a novel marker for the differentiation and progression of gastrointestinal cancers

Minireview

L. YU[1], L. WANG[1], S. CHEN[2]

[1]Department of Pathology, the First Affiliated Hospital, Sun Yat-sen (Zhongshan) University, Guangzhou, P.R. China; [2]State Key Laboratory for Biocontrol, Guangdong Key Laboratory of Pharmaceutical Functional Genes, Department of Biochemistry, School of Life Sciences, Sun Yat-sen (Zhongshan) University, Guangzhou, P.R. China, e-mail:lsschshw@mail.sysu.edu.cn

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Olfactomedin 4 (OLFM4) is a member of olfactomedin domain-containing protein family. Human OLFM4 is preferentially expressed in the gastrointestinal tract (stomach, small intestine and colon), prostate, and bone marrow. Recent studies demonstrate that OLFM4 is involved in the establishment and/or development of some types of malignancies, especially in gastrointestinal cancers. Induction of OLFM4 in cancer cells has a novel antiapoptotic action and promotes proliferation of cancer cells. OLFM4 regulates cell cycle and promotes S phase transition in proliferation of cancer cells. In addition, OLFM4 is associated with cancer adhesion and metastasis. In this minireview, we mainly focus on the OLFM4 expression and its biological significances in tumor differentiation and progression as well as the contributions of OLFM4 to tumorigenesis.

Key words: *olfactomedin 4, gastrointestinal cancer, tumor marker, tumor differentiation and progression, tumorigenesis*

Olfactomedin (OLFM) was first described in 1991 as a novel glycoprotein specifically expressed in the frog olfactory neuroepithelium [1], and its amino acid sequence showed no homology to any known protein [2]. Later research demonstrated that olfactomedin C-terminal shared an about 250 amino acids domain with many other proteins, which was named as "olfactomedin domain" [3]. There are at least 13 proteins containing the olfactomedin domain in mammals, and they play important roles in neurogenesis, neural crest formation, dorsal ventral patterning, cell-cell adhesion, cell cycle regulation, and tumorigenesis [4].

Olfactomedin 4 (OLFM4), also known as GW112 [5], human granulocyte colony-stimulating factor-stimulated clone 1 (hGC-1) [6], pDP4 [7], and hOlfD [8] in mammals, is a member of olfactomedin domain-containing protein family. OLFM4 gene, encoding 510 amino acids, was originally cloned from human hematopoietic myeloid cells [6]. Human OLFM4 is a secreted glycoprotein with N-linked carbohydrate chains and is able to form dimers and oligomers through disulfide-bond [9]. Cysteine 226 is essential for multimer formation. Mammalian OLFM4 shares 42% amino acids identities with frog Olfactomedin isolated from the olfactory epithelium.

OLFM4 gene was first found to be selectively expressed in the crypt epithelium of inflamed colonic mucosa [10]. OLFM4 mRNA is predominantly expressed in bone marrow, small intestine, colon and prostate [6]. OLFM4 protein expression has been observed in the oesophagus, stomach, small intestine and colon using immunohistochemistry [11]. In the recent years, increasing evidence indicates that OLFM4 is highly expressed in several types of tumors such as gastric [5, 12-15], pancreas [16], lung [16], breast [16] and colon [5, 16, 17] and is barely detectable in other tumors as well as in healthy tissues. OLFM4 expression is even related to tumor differentiation and progression and clinical course of diseases. In the present paper, we summarize the research progress of OLFM4.

Expression of OLFM4 and tumor differentiation and progression

Aberrant expression of OLFM4 has been detected in some cancerous tissues, especially in those of the digestive system [11, 14, 16, 18], suggesting that OLFM4 is a candidate gene for cancer-specific expression in patients with gastrointestinal cancer. The study based on serial analysis of gene expression (SAGE) indicates a relatively low level expression of OLFM4 in several normal tissues including prostate, breast, colon, and pancreas, but a high level expression of OLFM4 in the tissues of pancreatic, stomach, and colon cancers [5]. Abundant RNA transcripts of OLFM4 have been confirmed in those tissues of digestive system origin, rectum, colon, and stomach by Northern blot. OLFM4 mRNA was also detectable in breast cancer by Northern blot. In addition, an alternative splicing isoform was observed in breast, ovary, uterus, lung, kidney, and stomach cancers [5]. Using reverse transcription-polymerase chain reaction (RT-PCR), it has been demonstrated that OLFM4 mRNA is expressed in many human gastric cancer (GC) cells such as AGS, SNU5, SNU216, SNU620, SNU638, and SNU719 [19], and is up-regulated in 55% (22 of 40) of GC tissues [13, 14].

OLFM4 protein expression in tumor tissues is also detectable. Strong anti-OLFM4 immunoreactivity has been observed in the tumor tissues of a subset of colon, breast, and prostate cancers, whereas corresponding non-neoplastic glands are stained negatively or weakly for OLFM4 [20]. For example, 64% (21 out of the 33) colon cancers show strong reactivity. Out of the 5 breast cancers, 3 of them show strong anti-OLFM4 immunoreactivity. Among the 5 prostate adenocarcinomas tested, only 1 case shows strong, focal anti-OLFM4 reactivity [20]. The OLFM4 expression is enhanced in GC tissues demonstrated by immunostaining and 56% GC cases are positive for OLFM4 [11, 15].

Importantly, OLFM4 is supposed to be a useful marker for the differentiation and progression of tumors. Recent studies report a striking correlation between OLFM4 expression and the clinicopathologic features of patients with cancer in digestive system [11, 15, 21]. Enhanced OLFM4 expression is more frequently observed in intestinal-type gastric adenocarcinoma, whereas loss of expression tends to occur in the diffuse type [11]. OLFM4 is highly expressed in more differentiated gastric and colon cancers and is remarkably reduced or lost in poorly differentiated or undifferentiated gastric and colon cancers, suggesting that OLFM4 is a novel and sensitive marker for the differentiation of these tumors [11, 21]. OLFM4 immunostaining is observed more frequently in stage I/II GC cases than in stage III/IV GC cases [15] and OLFM4 expression is down-regulated in late tumor-node-metastasis stage, metastasis, and in colon cancer patients with shorter survival [21], indicating that reduced OLFM4 expression is associated with malignant progression of these gastrointestinal tumors. Interestingly, we search for candidate genes specific of endometrial adenocarcinoma through Digital Differential Display (DDD) and find that OLFM4 expression level in poorly differentiated endometrial adenocarcinoma is less than that in well-differentiated endometrial adenocarcinoma. This finding has been confirmed by OLFM4 immunohistochemical detection (Fig 1), which suggests a contribution of OLFM4 to differentiation of endometrial carcinoma.

Being a secreted glycoprotein that is associated with tumor progression, existence of OLFM4 in serum is of great interest. It has been demonstrated that the serum OLFM4 concentration in presurgical GC patients is significantly higher than that in healthy individuals, suggesting that serum OLFM4 is a useful marker for the early detection of GC [15].

What should be mentioned here is that OLFM4 may be a robust marker for stem cells in human intestine and marks

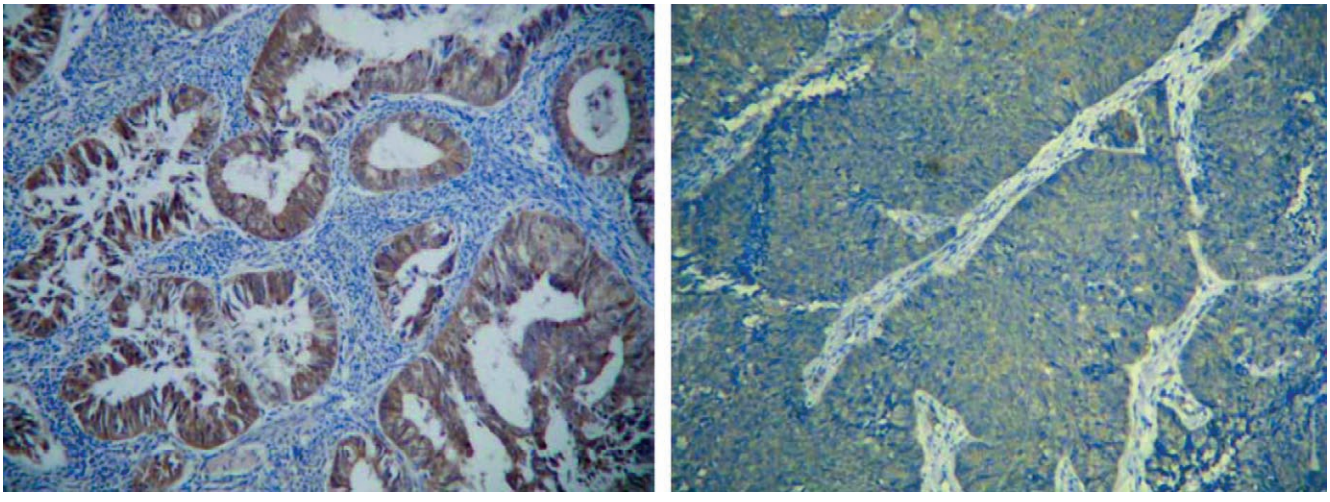

Figure 1. Expression of OLFM4 in endometrial adenocarcinoma detected by immunohistochemistry. Strong immunostaining was observed in well-differentiated endometrial adenocarcinoma (left), whereas a weak immunoreactivity was detected in poorly-differentiated endometrial adenocarcinoma (right).

a subset of colorectal cancer cells [22, 23]. Barker et al identify a group of stem cells defined by expressing G protein-coupled receptor Lgr5 in small intestine and colon [24], and Lgr5 stem cells represent the cells of origin of intestinal malignancies in the mouse [25]. Gene expression profiles of Lgr5 stem cells indicate that OLFM4 is a highly specific and robust marker for Lgr5 stem cells. High level of OLFM4 expression has been observed in crypt base columnar cells in human small intestine and colon [22]. Thus, expression of OLFM4 may be a useful marker to study the biology of stem cells in human small intestine and colon. Moreover, OLFM4 is highly expressed in subsets of cells from colorectal carcinomas and OLFM4 expression in these tumor cells is much higher than the expression observed in wild-type crypt base columnar cells at flanking crypt bottoms [22]. Since previous studies indicate that human colon adenocarcinomas harbor cancer stem cells [26, 27], OLFM4 may mark cells within colon adenocarcinomas that have such cancer stem cell properties.

Regulation of OLFM4 expression

The promoter of OLFM4 has been characterized and the regulation of OLFM4 expression in myeloid precursor cells [28] and gastric cancer cells [19] relies on nuclear factor-kappaB (NFκB). A thymidine residue 142 bp [19] or Adenine residue 24bp [28] upstream of the ATG initiation codon has been suggested to be a transcription start site through 5`-Rapid Amplification of cDNA Ends (RACE). The study by Chin et al has demonstrated that the 35bp region (-101 to -66) of the proximal promoter regulates OLFM4 expression in myeloid precursor cells [28]. The potential NFκB binding element, GGCCACTCCC, is located at -84 to -75. Mutation of the NFκB binding site within the promoter abolishes the binding of NFκB and its ability to regulate OLFM4 expression [28]. However, Kim et al identify a putative binding sequence of NFκB, GGTCTTTCC, between -442 and -430 region through sequence analysis of OLFM4 promoter [19]. 5`-deletion analysis indicates that positive regulatory element is located between -445 and -401. Deletion of the putative NFκB sequence shows consistently lower promoter activity, suggesting that this NFκB consensus sequence is required for basal expression of OLFM4 gene [19]. Overexpression of NFκB increases OLFM4 promoter activity in an NFκB sequence-dependent manner, and mutation of putative NFκB binding site in the OLFM4 promoter impairs response to the forced overexpression of p65, a subunit of NFκB [19]. The binding of NFκB to this putative site has been further confirmed by Electrophoretic Mobility Shift Assay (EMSA) and Chromatin Immunoprecipitation (ChIP) assay [19]. Moreover, OLFM4 mRNA and protein levels are induced by a forced overexpression of p65 and reduced by treatment of inhibitors for NFκB signal. The results strongly suggest that OLFM4 gene is regulated by NFκB signal.

OLFM4 was originally cloned from myeloid cells specifically induced by granulocyte colony-stimulating factor (G-CSF) [6]. G-CSF-induced OLFM4 expression in myeloid precursor cells is also regulated by NFκB [28]. Inhibition of the ERK pathway remarkably decreases G-CSF-induced OLFM4 expression, suggesting that G-CSF-induced expression of OLFM4 is mediated by the ERK MAPK signaling pathway [28]. In addition, it has been demonstrated that the ETS-family transcription factor PU.1 binds to a functional site within the promoter of pDP4, the murine homolog of human OLFM4, and regulates its expression in myeloid cells [7].

Roles of OLFM4 in tumorigenesis

It has been reported that induction of OLFM4 in cancer cells has a novel antiapoptotic action and promotes proliferation of cancer cells [5]. Overexpression of OLFM4 in mouse endothelial cell line SVEC significantly attenuates H_2O_2-induced apoptosis and inhibits H_2O_2-induced cytochrome c release and activation of caspase 3 and caspase 9 [5]. Antiapoptotic activity of OLFM4 may rely on its association with GRIM-19, a potent apoptotic inducer. OLFM4 association with GRIM-19 has been demonstrated by yeast two-hybridization and overexpression of GRIM-19 efficiently promotes retinoic acid/IFN-β induced apoptosis [5]. Cotransfection of plasmids expressing OLFM4 and GRIM-19 significantly attenuates GRIM-19-mediated, retinoic acid/IFN-β-induced cellular apoptosis. Forced expression of the GRIM-19 elevates the expression of apoptosis-related genes PIG12, GADD153, and c-Abl induced by retinoic acid/IFN-β, but expression of OLFM4 abolishes the up-regulation of gene expression induced by GRIM-19 expression [5]. Consistent with this result, a clonogenic assay indicates that GRIM-19 expression induces a significantly lower cell survival, but overexpression of OLFM4 gene facilitates survival of clonogenic tumor cell *in vitro* and tumor growth *in vivo* [5]. Taken together, these data suggest that OLFM4 is an important regulator of cellular apoptosis and plays important roles in tumor cell survival and tumor growth.

NFκB activity is up-regulated in many cancer cells [29] and facilitates tumor cell growth and resistance to a variety of cytotoxic agents [30], indicating the important role of NFκB in tumorigenesis. Interestingly, NFκB-induced OLFM4 expression promotes antiapoptotic activity of OLFM4 [19]. Overexpression or knock-down of OLFM4 gene in GC cells demonstrates that OLFM4 has an antiapoptotic property against the cytotoxic agents-induced apoptosis [19]. Proteasome inhibitor MG132 inhibits NFκB activation by preventing IκBα degradation, and treatment of GC cells with MG132 promotes cell apoptosis. Overexpression of OLFM4 attenuates MG132-induced apoptosis; in contrast, knock-down OLFM4 expression could increase MG132-induced apoptosis in GC cells [19]. These results indicate that OLFM4 could be an important mediator in NFκB-dependent tumorigenesis of gastrointestinal tissues and overexpression of OLFM4 may allow survival of GC cells in the presence of cytotoxic agents such as anticancer drugs [19].

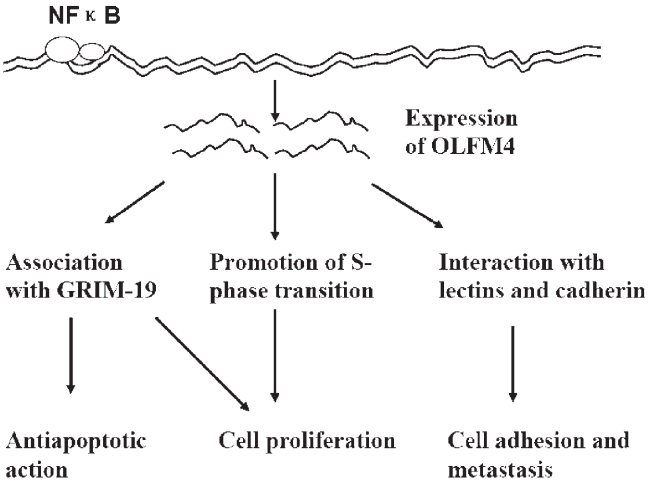

Figure 2. Potential roles of OLFM4 in tumorigenesis.

Moreover, involvement of OLFM4 in tumorigenesis may rely on its ability of regulating cell cycle. OLFM4 is expressed abundantly in pancreatic cancer tissue, and reduction of OLFM4 mRNA expression by small interfering RNA (siRNA) inhibits cell proliferation [18]. Interestingly, OLFM4 expression is especially elevated during early S phase of the cell cycle in pancreatic cancer cell cultures [18]. Silencing OLFM4 expression by siRNA decreases cell populations in G_1 phase and increases cell populations in S phase, demonstrating a typical cell cycle arrest at the S phase. Furthermore, cell volume enlarges without increase in multinucleated cells, supporting a premitotic inhibition of DNA synthesis. These data indicate that OLFM4 promotes cell proliferation by favoring transition from the S to G_2/M phase [18].

In addition, OLFM4 may regulate the tumor cell adhesion and migration. Purified OLFM4 as well as OLFM4-enriched culture supernatants enhance spreading and attachment of NIH3T3 and HEK293 cells [9]. As demonstrated by coimmunoprecipitation, OLFM4 binds to cadherin and lectins via its C terminal olfactomedin domain, implying that OLFM4, an extracellular matrix glycoprotein, may facilitate cell adhesion through a potential interaction with endogenous cell surface lectins and cadherin [9]. Forced expression of OLFM4 in human colon cancer cells changes cell morphology, actin distribution, and cell adhesion, suggesting that OLFM4 is involved in colon cancer adhesion and metastasis [21]. The roles of OLFM4 in tumorigenesis are summarized in Fig 2.

Conclusion

As a novel cancer related molecule, OLFM4 has been associated with tumor differentiation and progression. The roles of OLFM4 in tumorigenesis are likely due to its ability to regulate apoptosis, cell cycle and cell adhesion. However, the underlying mechanism that OLFM4 regulates tumor differentiation and cell survival remains further investigation. Since the most experimental data about OLFM4 expression available so far are limited to several organs of digestive system, expression profile of OLFM4 in a variety of organs and tumors should be investigated immediately. One important question to be answered is whether OLFM4 is a specific marker for gastroenteric tumor. If it is, how is it tissue-specifically regulated? Moreover, as a supposed secreted protein and putative biomarker, its significance in early diagnosis of tumors is of great interest. We believe that OLFM4 will become a potential target for therapeutic intervention in tumors, in particular those from gastrointestinal tract.

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