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ROLE OF CD10 IN THE METASTASIS OF COLORECTAL CANCER TO THE LIVER

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Abstract : CD10 is a widely expressed endopeptidase that is present in human colorectal cancer (CRC), which shows a high frequency of liver metastasis. CD10 expression in CRC cells is associated with liver metastasis in rodent models, and CD10 expression enhances the phosphorylation of epidermal growth factor (EGF) receptor (EGFR) and extracellular signal-regulated kinase (ERK) 1/2. Met-enkephalin (MENK), a CD10 substrate, activates its specific receptor δ-opioid receptor (DOR), which is expressed in CRCs. DOR is a partial agonist of ERK1/2, which suppresses EGF-induced phosphorylation of EGFR and ERK1/2. CD10 retains EGF-induced EGFR activation by degrading MENK. Paradoxically, CRCs express MENK at a high frequency. Since MENK suppresses T lymphocytes, CD10-expressing CRCs can escape from T-cell immunity without exhibiting auto-inhibition. CD10 is strongly associated with the metastasis of CRCs to the liver via an immunosuppressive mechanism. Additionally, CD10 may be an excellent serum marker for liver metastasis in patients with CRC and could represent a potential molecular target for antimetastatic treatment in patients with CRC.

Key words : CD10, Met-enkephalin, opioid receptor, EGFR

CD10

Colorectal cancer (CRC) is the fourth leading cause of cancer-related death in Japan, which is increasing as the Western lifestyle is becoming more popular in Japanese populations [1]. Approximately 24% of CRC invading beyond the submucosal layer shows liver metastasis at the time of the original operation and/or during the follow-up period after operation [2]. One third of patients with CRC die from liver metastasis [3], and only approximately one-third of patients with CRC with liver metastasis will respond to systemic chemotherapy; indeed, long-term survival is rare [3]. Early detection and control of liver metastasis are important factors in the treatment of patients with CRC.

CD10, also known as common acute lymphoblastic leukemia antigen (CALLA), is widely used to define subgroups within B cell-type acute lymphocytic leukemia [4;5]. CD10 is a 90- to 110-kDa cell zinc-dependent membrane metalloendopeptidase, also referred to as neutral endopeptidase 24.11 (EC 3.4.24.11), enkephalinase, or neprilysin [5]. CD10 is widely expressed in various tissues,

including granulocytes, lymphoid germinal centers, lymphoid progenitor cells, placental syncytiotrophoblasts, renal tubules, glomeruli, mucosal epithelium of the bronchus, stomach, intestine, salivary glands, prostate, and gallbladder [6-8]. Substrates of the enzyme include a wide range of neuropeptides, such as substance P, bradykinin, bombesin, Leu-enkephalin (LENK), and Met-enkephalin (MENK) [5; 9; 10].

CD10 is expressed in CRC and is associated with CRC metastasis, particularly liver metastasis [2; 11; 12]. Invasion deeper than the subserosa, venous invasion, lymph-node metastasis, and CD10 expression are significantly associated with CRC liver metastases [2]. Logistic regression analysis showed that lymph node metastasis, CD10 expression, and vascular endothelial growth factor (VEGF) expression were significant factors associated with CRC liver metastasis and were independent of the incidence of vascular invasion, expression of CD44, and expression of transforming growth factor (TGF)- α [12]. CD10 expression in cancer stromal cells is associated with CRC invasion and metastasis [13]. Moreover, CD10 is expressed in the normal mucosa of the small intestine but not in the colorectum [14]; however, the functions of CD10 in the normal intestinal mucosa and CRC are still unknown.

CD10 enhances liver metastasis

The role of CD10 expression as a surrogate marker or biological activator of disease progression has not yet been defined. In our previous studies, we confirmed that the CD10-positive human colon cancer cell line HT29 [15] showed inhibition of cell growth, invasion, and colony formation following treatment with CD10 antisense S-ODN. In a mouse liver metastasis model, HT29 cells treated with CD10 antisense S-ODN showed fewer embedded cells in the liver than control HT29 cells. The numbers and sizes of metastatic foci in the livers of nude mice were reduced in mice inoculated with CD10 antisense S-ODN-treated HT29 cells [15]. Intraperitoneal administration of liposome-capsulated CD10 antisense S-ODN inhibits the establishment of liver metastasis and the growth of established metastases in nude mice [15].

CD10 substrates

Substrates of CD10 include a wide range of neuropeptides, such as substance P, bradykinin, bombesin, LENK, and MENK [5; 9; 10]. Among these neuropeptides, substance P and enkephalin participate in mitogen-activated protein kinase (MAPK) signaling [16; 17]. Moreover, substance P transactivates epidermal growth factor (EGF) receptor (EGFR) [18], whereas enkephalin-induced EGFR transactivation is dependent on integrin signaling [19]. In G6 glioma cells, which express enkephalin receptor (δ opioid receptor [DOR]) and EGFR, EGFR activation is inhibited by activated DOR, although EGFR does not inhibit DOR [20]. Thus, enkephalin degradation by CD10 may promote cancer through activation of EGFR. Tumor growth is a balance of cell proliferation and apoptosis. CD10 may affect signal diversity during MAPK activation by EGFR to increase cell growth and decrease apoptosis. Subsequently, CD10 enhances tumor growth and metastasis.

CD10-related intracellular signal pathways

MAPK activation is known to mediate cell growth, invasion, and survival. Thus, in our previous work, we examined the effects of CD10 repression on MAPK phosphorylation [15]. CD10 antisense S-ODN treatment increased extracellular signal-regulated kinase (ERK) 1/2 phosphorylation, which is associated with cell growth and invasion [21]. Treatment with CD10 antisense S-ODN decreased the phosphorylation of ERK1/2 and EGFR in HT29 cells [15]. In contrast, CD10 antisense S-ODN treatment decreased p38 phosphorylation. Moreover, we confirmed that p38 inhibition decreased apoptosis in HT29 cells. EGFR is a major activator of MAPK in CRCs [22]. Therefore, we examined the effects of CD10 antisense S-ODN treatment on EGFR activation. CD10 antisense S-ODN treatment decreased EGFR phosphorylation, suggesting that CD10 may be associated with EGFR activation.

In our recent studies, we demonstrated that DOR is a high-affinity receptor of MENK and belongs to the family of the G-protein-related receptors [17]. We examined the activation of MAPK proteins in HT29 cells and found that the level of ERK phosphorylation in EGFR(+)/DOR(–) cells was higher than that in EGFR(+)/DOR(+) cells. This finding was supported by a report suggesting that signals from both EGFR and DOR are transduced via the MAPK pathway and that DOR activation modulates EGFR activation and ERK1/2 phosphorylation [23]. The findings that EGFR knockdown increases the phosphorylation of DOR and that DOR knockdown increases the phosphorylation of EGFR suggest that EGFR and DOR interfere with the activation of one another. Both DOR and EGFR recruit G-proteins for intracellular signaling; hence, their signals may interfere with each other [23]. Additionally, the expression of DOR and EGFR in G6 glioma cells has been studied; activation of DOR inhibits the subsequent activation of EGFR, whereas activation of EGFR does not inhibit the activation of DOR [20]. GRK2, a G-protein, enhances the internalization of DOR but not EGFR [24]. Thus, further studies are needed to confirm the relationship between DOR and EGFR.

Host anticancer immunity and CD10

Morphine can decrease the effectiveness of several functions of both natural and adaptive immunity and can also significantly reduce cellular immunity [25]. The differentiation function of immune cells is significantly affected by opioids [26]. In animal studies, morphine is consistently associated with increased morbidity and mortality and promotes cancer progression [25]. Chronic administration of opioids decreases the proliferative capacity of macrophage progenitor cells and lymphocytes. T-lymphocyte responses are depressed by morphine, as assessed by the inhibition of delayed-type hypersensitivity reactions and cytotoxic T-lymphocyte activity, modulation of T-lymphocyte antigen expression, and depression of responses to T-lymphocyte mitogens [27].

MENK is a neuropeptide that exhibits opium-like effects on the central nervous system. Morphine affects the immune system and inhibits cellular immunity [25]. Opioids alter the second messenger cyclic AMP, intracellular calcium, and kinases activated by second messengers in immune cells [28; 29].

Similar to morphine, MENK is an immunomodulator that modifies immune responses

to extracellular stimuli, such as mitogens and antigens [30]. DOR, the specific receptor of MENK, is expressed in T lymphocytes [30]. In some contexts, opiates induce apoptosis of T lymphocytes through the c-Jun N-terminal kinase (JNK) pathway [31]. MENK is produced in high concentrations in colon cancer [32], and MENK secretion is associated with tumorigenicity and metastasis of CRC cells in syngeneic rodent models [33]. MENK concentrations in subcutaneous tumors of CT26 and IEC6A rodent CRC cells are inversely correlated with the number of tumor-infiltrating T lymphocytes. MENK inhibits the growth of MOLT-4 T-lymphoblastic cells in a concentration-dependent manner. Furthermore, MENK increases the phosphorylation of JNK and induces apoptosis in MOLT-4 cells. MENK-induced apoptosis is abrogated by treatment with a JNK inhibitor.

The expression of MENK, DOR, and CD10 was examined in 61 human CRCs by immunohistochemistry [34]. All tumors expressed MENK. Moreover, DOR and CD10 expression was detected in 13 (21%) and 17 (28%) of the 61 cases. Eleven (73%) of the 15 Dukes' D cases with liver metastasis concurrently expressed MENK, DOR, and CD10, whereas two (10%) of the 21 Dukes' C cases and none of the Dukes' B cases showed co-expression of the three genes ($P < 0.0001$). MENK expression is associated with Dukes' staging, nodal metastasis, and liver metastasis. MENK concentrations in tumor tissues are higher in Dukes' C cases than in Dukes' B cases. Moreover, MENK expression is associated with tumor-infiltrating T lymphocytes, particularly those belonging to the $CD4^+$ subset [33]. DOR is expressed in phytohemagglutinin-stimulated $CD4^+$ and $CD8^+$ T lymphocytes and modulates T lymphocyte proliferation, IL-2 production, chemotaxis, and intracellular signaling [35]. Exposure to a pure DOR antagonist results in dose-dependent suppression of concanavalin A-induced rat T-lymphocyte proliferation [36].

In CRCs, the anticancer immunity of the host is suppressed due to the secretion of several cytokines. High-mobility group box 1 (HMGB1) protein induces apoptosis in macrophages [37], thereby reducing the numbers of tumor-associated macrophages and enhancing the metastasis of CRCs [38]. Programmed death-1 (PD-1) is an inhibitory receptor expressed in T lymphocytes. The expression of PD-1 ligand by CRC cells induces apoptosis in tumor-infiltrating T lymphocytes [39]. Serum receptor-binding cancer antigen expressed on SiSo cells (RCAS1), a membrane molecule expressed on human cancer cells, suppresses tumor-infiltrating T lymphocytes by inducing apoptotic cell death [40; 41]. These factors cause the escape of cancer cells from host immunity, thereby enhancing disease progression and metastasis. MENK is thought to be one of the primary immune evasion factors in CRCs.

DOR, a receptor with high affinity for MENK, is a member of the G-protein-related receptor family [17]. DOR transmits intracellular signals through heterotrimeric G proteins to the family of MAPKs [42]. In DOR-transfected Jurkat cells, DOR agonists stimulate MAPK phosphorylation in a Ras-independent and protein kinase C (PKC)-dependent manner [43]. The diversity of DOR activity depends on the multifunctionality of MAPK, which is attributed to the activation profiles of MAPK family members [44]. DOR and κ -opioid receptor (KOR) are expressed in T lymphocytes with a similar expression pattern to that observed in MOLT-4 cells [45; 46]. Notably, in MOLT-4 cells, MENK suppresses cell growth in a concentration-dependent manner and decreases the phosphorylation of ERK1/2 and p38, but increases the phosphorylation of JNK.

These results are supported by the findings of a study by Singhal et al. [31], in which DOR-induced JNK activation was found to be associated with T-lymphocyte apoptosis. Additionally, ERK phosphorylation also attenuated T lymphocyte activation through DOR, which is expressed by activated T lymphocytes [47].

With the exception of MAPK activation, DOR affects the activation of T lymphocytes in all other intracellular events. DOR activation affects intracellular calcium concentrations, and DOR agonists increase intracellular free calcium concentrations, whereas MOR is not associated with calcium concentrations [48]. In contrast, DOR is negatively coupled with adenylate cyclase for the production of cyclic AMP [28]. DOR also plays a role in transcriptional regulation by activating activator protein (AP)-1, AP-2, c-fos, Ikaros-1, Ikaros-2, nuclear factor-kappaB, and activating transcription factor-2 in immune cells, including T lymphocytes [29; 49]. Ikaros enhances *DOR* gene expression transcriptionally in phytohemagglutinin-activated T lymphocytes [50]. Thus, DOR may provide negative feedback for T-lymphocyte activation through several intracellular signaling mechanisms.

CD10 as a serum marker for metastasis of CRC to the liver

Liver metastasis is a major life-threatening condition in patients with CRC [2]. Early detection and treatment are essential for the management of liver metastasis [51]. Early detection of liver metastasis involves detection of metastasis using imaging or serum markers and prediction tools to identify patients who are at high risk of liver metastasis [52]. For example, liver metastasis has been shown to be highly associated with serum carcinoembryonic antigen levels [53]. Overexpression of sialyl Lewis X, c-erbB2, and c-met is associated with higher malignant potential in liver metastasis [54-56]. However, these factors have not been shown to have high sensitivity and/or specificity to predict liver metastasis in patients with CRC. As described above, CD10 is an excellent histological marker for liver metastasis of CRC. If CD10 can be detected in the serum, this protein may have clinical importance.

CD10 is expressed at the luminal surface of endothelial cells, the small intestine, and many other organs [14; 57]. CD10 is localized mainly on the apical plasma membrane; however, we detected CD10 in the stromal space among cancer nests, which suggests that CD10 is secreted from CRC cells [34]. Increased levels of CD10 are reported in allergic diseases such as bronchial asthma or hemodialysis conditions [58] [59]. These findings suggest that serum CD10 might be a possible marker for the liver metastasis in CRCs.

In a mouse subcutaneous tumor model, serum CD10 is correlated with tumor weights [60]. Serum CD10 levels were examined in 84 patients with CRC and were shown to be higher in patients with more advanced cancer [60]. Patients with liver metastasis showed the highest levels of serum CD10 among all patients. Moreover, patients with high serum CD10 levels were found to have had metachronous liver metastasis [60].

A cutoff of serum CD10 set to greater than 1000 pg/mL showed 70% sensitivity and 93% specificity for liver metastasis in CRC[60]. This cutoff included all cases of metachronous liver metastasis. After excluding related conditions, such as allergy or hemodialysis, serum CD10 levels may serve as a useful marker of synchronous and metachronous liver metastasis in CRC.

CD10 targeting

Since CD10 plays a role in prometastatic mechanisms by affecting host antitumor defense through degradation of enkephalin or angiotensin, CD10 is a good candidate for molecular targeting therapy. We have previously reported that CD10 knockdown inhibits liver metastasis of CRC cells using a mouse model [15]. Racecadotril, an antirelaxant drug, and thiorphan and AHU377, which are used as antihypertensives, are anti-CD10 drugs [61-63]; application of these drugs to CRC treatment in large clinical trials may facilitate the identification of effective antimetastatic strategies in CRC.

Conclusion

MENK exhibits different effects in various cell lineages, including induction of cell survival and proliferation in neural cells [64], suppression of T cells [30; 31; 65], and growth inhibition in epithelial cells [66; 67]. Different responses of MAPK families to MENK may be responsible for the diversity of MENK function. In CRC, T-cell suppression is induced by MENK secretion, whereas the degrading activity of CD10 on MENK may permit HT29 cell escape from MENK-induced growth suppression and apoptosis.

Two clinical applications of CD10 have been proposed. First, CD10 may be an excellent serum marker for liver metastasis in patients with CRC. Assessment of serum CD10 levels may enable the selection of patients who are at high risk of metachronous liver metastasis of CRC, which could facilitate the administration of preventive chemotherapy before the diagnosis of liver metastasis. Second, CD10 is a possible target for antiprogressive and antimetastatic treatments in CRCs. We observed the antimetastatic effects of the CD10 inhibitor thiorphan with systemic administration in mice. Antitumor effects have been reported in the literature [68]. Racecadotril, which is rapidly metabolized to its active metabolite thiorphan, is already used in clinical practice for pediatric diarrhea and is considered safe [61]. Concurrent administration of a CD10 inhibitor with chemotherapeutic agents should be evaluated to determine its antimetastatic effects in colon cancer treatment.

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