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学 位 論 文 名	Overexpression of endogenous TIMP2 increases the proliferation of BeWo choriocarcinoma cells through the MAPK signaling pathway （内因性のTIMP2の過剰発現により、MAPKシグナル伝達経路を介したBeWo細胞増殖が亢進する）。
論文審査委員	（主査） 教　授　木村　　正 （副査） 教　授　金倉　　譲　　教　授　野々村　祝夫

論 文 内 容 の 要 旨

〔目　的(Purpose)〕

Choriocarcinoma is a highly malignant form of trophoblastic tumor that is characterized by malignant placental tumors and rapid cell growth. *In vivo* and *in vitro* studies have demonstrated that tissue inhibitor of metalloproteinase 2 (TIMP2) is present in choriocarcinoma. However, the role of TIMP2 in cell proliferation in choriocarcinoma has not been investigated. Exogenous TIMP2 is known to promote cell proliferation. During growth, cells are subjected to varied concentrations of TIMP2, which depend on the amount of TIMP2 produced by the cells themselves. Thus, the effect of gradually increasing endogenous TIMP2 on the proliferation of choriocarcinoma cells needs to be examined.

〔方法ならびに成績(Methods/Results)〕

TIMP2 expression plasmid (pLV-*TIMP2*) was transfected to the BeWo human choriocarcinoma to evaluate effect of endogenous TIMP2 on cell proliferation. pLV-*EGFP* (enhanced green fluorescent protein) was used as control. We also used HEK293T (human embryonic kidney cell line) and HT-1080 (human fibrosarcoma cell line) as controls to compare the characteristic of BeWo cells in terms of response to the biologically relevant condition of TIMP2. To evaluate the effect of endogenous TIMP2 on cell proliferation, we first counted the number of cells with a hemacytometer. 48 hours after transfection, number of BeWo cells was significantly higher in the group transfected with pLV-TIMP2 than the control group ($P < 0.05$). As for the HT-1080 and HEK293T cells, there was no significant difference between the groups. To confirm, we examined the cellular DNA synthesis rates by using a BrdU cell proliferation kit. The results again showed that cells transfected with the *TIMP2*-expressing plasmid had significantly higher growth rates than the control group in the BeWo cell line ($P < 0.05$) but no difference in the HT-1080 or HEK293T cell lines ($P > 0.05$).

During implantation and cancer development, MMPs and their inhibitors (TIMPs) are known to play major roles in mediating and regulating the invasion and remodeling of the extracellular matrix of tissue. In addition, MMP2 and MMP9 were recently demonstrated to influence cell proliferation. Therefore, we hypothesized that MMP2 and MMP9 play a role in proliferation in our study. However, the gelatin zymography analysis revealed that the activities of MMP2 and MMP9 were not affected by TIMP2 overexpression in any of the 3 cell lines. Therefore, the MMP2 and MMP9 systems are not involved in the TIMP2-induced proliferation of BeWo cells.

Previous studies have shown that two main signaling pathways are involved in exogenous TIMP2-induced cell proliferation: the MAPK pathway and the cyclic-AMP pathway. Western blot was used to measure the phosphorylation of extracellular

signal-regulated kinase-1/2 (p-ERK1/2) and c-Jun N-terminal kinase-1/2 (JNK 1/2). We found that by inducing overexpression of endogenous TIMP2, activation of ERK1/2 and JNK1/2 of the MAPK signaling pathway was significantly higher than the control group in BeWo cells ($P > 0.05$), but there was no significant difference in either HT-1080 or HEK293T cells. Cyclic AMP was measured by a competition enzyme-linked immunoassay. We found that cAMP had a tendency to increase in BeWo cells compared with the level in the control; however, similar to HT-1080 and HEK293T cells, there was no significant difference compared with the control group in this regard ($P > 0.05$).

Furthermore, to confirm the role of ERK 1/2 and JNK 1/2 in endogenous TIMP2-induced BeWo cell proliferation, we used a specific cell-permeable inhibitor of ERK kinase (PD 98058) and a selective JNK inhibitor (SP600125) to inhibit the MAPK signaling pathway before pLV-*TIMP2* transfection. The results showed that activation of ERK1/2 and JNK 1/2 was significantly inhibited, and that the effect of TIMP2 on BeWo cell proliferation was also inhibited.

〔総　括(Conclusion)〕

In conclusion, this study showed that gradual increase in endogenous TIMP2 could stimulate cell proliferation in BeWo cells through upregulation of the MAPK pathway. This result suggests that TIMP2 presented in choriocarcinoma is not only important in mediating invasion as reported in previous studies but also important in the growth of choriocarcinoma in the biological condition. Moreover, the proliferation of BeWo cells due to TIMP2 can be used as a model of fast-growing choriocarcinoma, and TIMP2 could be used as a promising footstep or a novel tumor marker of choriocarcinoma. To confirm this achievement, more data on studies involving humans are required.

論文審査の結果の要旨

Tissue inhibitor of metalloproteinase 2(TIMP-2) は、matrix metalloproteinase群の阻害剤として有名であるが、その一方で、細胞増殖を促進することも報告されている。しかし、今までの報告は、培養細胞を一定濃度のTIMP-2に曝された状態での実験であった。今回、TIMP-2を3種類のcell lines(絨毛癌由来のBewo cells、ヒト繊維肉腫細胞由来のHT1080 cells、ヒト胎児腎臓由来のHEK293Tcells)にtransfectionし、徐々にTIMP-2濃度が上昇する系を作製した。この系においては、BeWo細胞のみがコントロールであるGFP transfection群と比較して細胞が有意に促進された。また、この増殖はMAPK-signaling pathwayを介したものであることを示した。絨毛癌の組織がTIMP-2で染まるという他のグループの報告と合わせて、TIMP-2が絨毛癌の増殖に関与している可能性が示唆された。

この研究は今後の絨毛癌の研究に大きく貢献し得るものであり、学位の授与に値すると考えられる。