主論文の要旨

Urokinase-type plasminogen activator receptor promotes proliferation and invasion with reduced cisplatin sensitivity in malignant mesothelioma

（ウロキナーゼ型プラスミノーゲン活性化因子受容体は悪性中皮腫の増殖・浸潤を促進しシスプラチン感受性を低下させる）

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<Background>

Malignant mesothelioma (MM) arises from the serosal mesothelial cells of somatic cavities and is an aggressive neoplasm. Exposure to asbestos is the primary cause of MM. The incidence of MM is increasing worldwide. Chemotherapy, surgery and radiation are rarely curative for MM. Therefore, it is critical to identify key molecules for the early diagnosis of MM and development of novel therapies.

Urokinase-type plasminogen activator receptor (uPAR; *Plaur*), is a glycosyl-phosphatidylinositol (GPI)-anchored membrane protein. It was originally identified as a cell-surface binding site for urokinase-type plasminogen activator (uPA; *Plau*). Recent studies suggested that uPAR can act as a signaling receptor in cooperation with transmembrane receptors to activate major intracellular signaling pathways, such as the phosphatidyl inositide 3-kinase (PI3K)/AKT pathway. *uPAR* has been demonstrated as a component of the main systems involved in the growth, metastasis and angiogenesis of several solid and hematologic malignancies. Moreover, elevated plasma levels of its cleaved form, called soluble uPAR, are frequently associated with poor prognosis in breast and colorectal cancers. Here, for the first time, we measured and modulated *uPAR* expression in asbestos-induced MM tissues and cells to determine downstream signaling alterations. We also investigated its impact on chemotherapy and examined the serum uPAR levels in MM patients.

<Materials and methods>

Rat MM tissue array and whole-genome microarrays (G4131F; Agilent Technologies, USA) were used; two normal mesothelial tissues obtained by scraping normal rat pleural and peritoneal cavities were used as controls, and 21 rat MM tissues [8 epithelioid (EM) and 13 sarcomatoid subtype (SM)] were used. Rat MM cell lines (EM 1-5 and SM 1-5) were established from the ascites of mesothelioma-bearing rats models. We also used 8 human MM cell lines. Rat peritoneal mesothelial cells (RPMC) and immortalized human mesothelial cells, MeT-5A were used as controls. The APOAF Annexin V apoptosis kit (Sigma, USA) was used and $AnnexinV^{high}/PI^{low}$ cells were recognized as early apoptosis and $AnnexinV^{high}/PI^{high}$ cells were identified as late apoptosis or necrosis. A human mesothelioma tissue array was purchased (Biomax, USA). A human uPAR quantikine ELISA kit (R&D Systems, USA) was used with the human serum samples.

<Results>

Based on our previous data of asbestos-induced MM (GSE48298) in rats, both histological subtypes of MM showed approximately 6-7-fold increase in *uPAR* expression compared with scraped normal mesothelial cells (Fig. 1A-C). And strong *uPAR* expression in rat MM was associated with significantly shorter survival during carcinogenesis (Fig. 1D-E). Most of the rat and human MM cell lines also showed elevated expression of *uPAR* (Fig. 1F-G).

In both the EM and SM cell lines, stable *uPAR* knockdown by *uPAR*-targeted *sh*RNAs resulted in significantly suppressed proliferative, migratory and invasive properties of the rat MM cells (Fig. 2A-D). Conversely, we overexpressed *uPAR* in a human MM cell line with low *uPAR* expression, Y-Meso-8A, using a human *uPAR* expression vector. *uPAR* overexpression stimulated proliferation, migration and invasion in Y-Meso-8A cells (Fig. 2E-H).

To further evaluate alterations in intracellular signaling after modulating *uPAR* expression, we observed that AKT/mTOR activity was closely associated with *uPAR* expression, as demonstrated through *uPAR* knockdown and overexpression in MM cells (Fig. 3A-B). Moreover, *uPAR* knockdown significantly impeded MM cell growth and AKT activity in xenografts (Fig. 3C-D).

Furthermore, *uPAR* knockdown sensitized both EM and SM cells to cisplatin (CDDP) at 24 h (Fig. 4A). After CDDP treatment with *uPAR* knockdown, the proportion of early/late apoptotic cells increased significantly (Fig. 4B-C). It suggested that caspase-3 cleavage increased when CDDP suppressed the AKT pathway, which was promoted by *uPAR* knockdown, and these effects were nearly abolished by sustained AKT activation (Myr-AKT transduction) (Fig. 4D).

In addition, increased uPAR was observed in eight MM tissue cores from four patients, and pAKT showed corresponding alterations compared with benign pleural lesions in a human MM tissue array (Fig. 5A). Based on the analyses of the control group, including 12 participants without malignant disease, versus the group of MM patients [N=21; 15 EM and 6 biphasic subtype (BM)] and 13 patients with lung cancer, we observed significantly increased serum uPAR levels in the MM patients (Fig. 5B).

<Discussion>

Here, for the first time, we showed that *uPAR* overexpression is observed in asbestos-induced rat MM, regardless of the asbestos fibers used for carcinogenesis and the histological subtype. Moreover, we found a significant correlation between survival and *uPAR* expressions in rat models. These data indicate that *uPAR* overexpression is a common and important expressional alteration in MM. Exposure to asbestos has been reported to upregulate *uPAR* expression in mesothelial cells. Therefore, the link between this first reaction to asbestos and carcinogenesis highlighted the involvement of *uPAR* and it would be an interesting subject for future investigations.

Indeed, *uPAR* is overexpressed in some human cancers, including breast, gastric and lung cancer. Coincidently, *uPAR* overexpression was mostly reproduced in both rat and human MM cell lines, and with knockdown and overexpression studies *in vitro* and *in vivo*, we showed that *uPAR* is intimately associated with the malignant character of MM cells.

Moreover, we for the first time showed the association between *uPAR* overexpression and downstream PI3K/AKT/mTOR signaling with *uPAR* modulation in MM cells and xenograft tumors, as well as in human MM tissue samples. The PI3K/AKT pathway has been identified as

a key regulator of survival during cellular stress. Therefore, induced activation of AKT downstream genes by *uPAR* overexpression in MM can be considered a critical target for future MM treatment.

Our results also showed that MM cells revealed caspase activation and apoptotic features with cisplatin treatment, which were inhibited by *uPAR* overexpression and promoted by *uPAR* knockdown. We found that the sensitivity of MM cells to cisplatin is related to *uPAR* induced AKT activation. To the best of our knowledge, this is the first report of *uPAR* expression being associated with cisplatin sensitivity through the AKT pathway in MM.

Cleaved uPAR from the MM cell membrane leads to uPAR secretion into the blood flow, and our study was the first to show that MM patients have higher serum uPAR levels compared to those with benign diseases. The serum uPAR level is closely associated with its expression and has been suggested as a prognostic biomarker in some cancers (e.g., ovarian and prostate cancer). With the ease of testing serum uPAR levels, our results suggest that the serum uPAR level could serve as a marker for MM diagnosis and therapy monitoring.

<Conclusion>

We showed that elevated *uPAR* expression in MM increases AKT signaling activity, which is a major regulator of cisplatin-induced apoptosis.

Abbreviations: uPA, urokinase-type plasminogen activator; uPAR, urokinase-type plasminogen activator receptor; EM, epithelioid subtype mesothelioma; BM, biphasic subtype mesothelioma; SM, sarcomatoid subtype mesothelioma; MM, malignant mesothelioma; CDDP, cis-diamminedichloroplatinum; RPMC, rat peritoneal mesothelial cell.