Calreticulin: a critical inducer in metastasis of esophageal squamous cell carcinoma

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Esophageal squamous cell carcinoma (ESCC) is one of the most common malignancies worldwide, and metastasis is responsible for most of the cancer deaths [1]. However, the mechanism of ESCC metastasis is not well understood.

In our previous study, we have shown that overexpression of calreticulin (CRT) correlated with poor prognosis for patients with ESCC [2]. CRT is a critical regulator in protein synthesis and Ca2+ signaling, mainly residing in the lumen of the endoplasmic reticulum (ER). Klampfl et al. have recently reported that CRT was somatically mutated in non-mutated JAK2 essential thrombocythemia and primary myelofibrosis [3]. We performed whole-genome sequencing in 17 ESCC cases and whole-exome sequencing in 204 cases, and targeted deep sequencing in 119 cases of ESCC, but have not found the existence of CRT mutation in this disease [4-6]. Interestingly, CRT has been observed to be over-expressed and promote metastasis in multiple types of cancers [7]. We found that knockdown of CRT decreased cell migration, invasion, anoikis resistance and tumor formation in nude mice. CRT regulates the transcription of cortactin (CTTN) through STAT3 [8]. We have reported CTTN as an oncogene by promoting cell migration and anoikis resistance via the PI3K-AKT pathway [9]. We have also shown that ectopic expression of CTTN rescued the defects of cell motility and anoikis resistance in CRT down-regulated ESCC cells [8]. These data indicate a novel signaling pathway, CRT-STAT3-CTTN-AKT in promoting ESCC cell motility.

To identify the cellular mechanism of tumor metastasis induced by CRT, we performed a cDNA array to analyze gene expression patterns by CRT regulation and showed several genes, including protein-tyrosine phosphatase 1B (PTP1B) and neuropilin 1 (NRP1), down-regulated in CRT-siRNA cells, further confirmed by real-time PCR and western blot analysis [10].

PTP1B is a widely expressed non-transmembrane protein tyrosine phosphatase that acts as an important regulator of multiple signaling pathways and plays a tumor promoter or suppressor depending on in different cancers [11]. We found that PTP1B promotes cell motility and tumor progression, as
a downstream effector of CRT in ESCC $^{[10]}$. At the molecular level, CRT regulates the transcriptional activity of STAT5A, and PTP1B expression is modulated by STAT5A. CRT and PTP1B promote ESCC cell motility through ERK1/2. Notably, PTP1B expression positively correlates with CRT in primary ESCC tissues and is associated with a poor prognosis in CRT over-expressed ESCC patients, suggesting another signaling pathway, CRT-STAT5A-PTP1B-ERK1/2, involved in ESCC metastasis.

Very recently, we confirmed NRP1 as a new downstream effector of CRT $^{[12]}$. NRP1 is a single-pass transmembrane receptor, which participates in several different types of molecular pathways that control cell migration. It is reported that increased expression of NRP1 correlates with metastasis in various tumor types $^{[13]}$. We found that NRP1 contributes to ESCC cell motility in vitro and experimental metastasis in vivo. CRT promotes NRP1 transcription still via STAT5A. We also observed that some metastasis-related proteins were down-regulated in CRT or NRP1-silenced ESCC cells, including MMP9, MMP2 and FAK. Furthermore, CRT and NRP1 co-expression significantly correlated with lymph node metastasis of ESCC patients. Therefore, we identified a novel molecular mechanism of ESCC progression enhanced by CRT overexpression.

Taken together, we revealed that CRT is an important regulator in metastasis of ESCC. However, the regulatory mechanism among the above-mentioned molecules remains unclear (dotted lines in Figure 1), which is worth further investigating. First, how does CRT regulate the transcriptional activity of STAT3 or STAT5A? How does CRT or CTNN modulate AKT phosphorylation? How does PTP1B promote ERK1/2 activity? Moreover, by which molecules does NRP1 regulate the metastasis-related proteins MMP9, MMP2 and FAK? Finally, can CRT signaling be used as a molecular target for ESCC treatment? Addressing these issues will enlarge our view of CRT in ESCC metastasis.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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