# Orchestration of the crosstalk between astrocytes and cancer cells affects the treatment and prognosis of lung cancer sufferers with brain metastasis

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**Abstract:** Brain metastasis is an inauspicious consequence of lung cancer. However, the majority of cancer cells that seep into the brain died of unknown causes, only a few survived and developed into metastatic brain tumor. Communication between cancer cells and host tissue is viewed as an essential event during metastasis, but little is known about the accurate control of this processes. Within the lesion of brain metastasis, abundant activated astrocytes are observed with lung cancer cells. Previous studies have demonstrated that the astrocyte network served a protective role in the central nervous system (CNS) and most malignant cells that seep into the brain perish were rejected by astrocytes. Reactive astrocytes generated protease plasmin and cytotoxic cytokines as a defense against metastatic invasion. But recently, other investigators argued that tumor cells interactions with astrocytes promote the progression of brain metastases and protect them from the cytotoxic effects of chemotherapy. In this article, we review the architecture between astrocytes and infiltrated cancer cells, and raise a future perspective on therapeutic potential of targeting crosstalk modulators against brain metastasis.

**Keywords:** Brain metastasis; astrocytes; lung cancer; chemoresistance

## Introduction

Lung cancer is one of the most common malignant tumors and remains the leading cause of cancer-related lethality worldwide (1,2). As the common site of extra-thoracic distant metastasis of lung cancer, brain is most reported for the poor prognosis (3). Multistage steps are implicated in the establishment of clinically relevant brain metastasis (4). The classical *seed and soil* hypothesis enlightens that the successful growth of metastatic cells are dependent on the interactions and characteristics of cancer cells (seeds) and their target organs (soil) (5). As it explicates, circulating cancer cells must overcome many obstacles to colonize in brain (6). However, once the distinctive biology of metastases is established in brain, the host microenvironment will play a "sanctuary" role in promoting metastatic growth and treatment resistance (7), and then current treatments frequently fail to provide durable responses (8). An improved understanding of the crosstalk between microenvironment and cancer cells is needed to prevent and treat metastatic cancer (9).

Astrocytes participate in the repair and scarring of the brain following injuries and are pretty crucial for microenvironment homeostasis. Dysregulation of astrocytes is thought to contribute to the pathogenesis of brain metastasis progression (10). In brain metastases, an abundance of activated astrocytes were found within the lesion (11). Cancer cells that infiltrate the brain are

immediately exposed to astrocytes in the perivascular space and produce deleterious signals to repel invading cells (6). Cancer cells must be shielded from such signals in order to survive and to extract benefits from the stroma, including benefits from astrocytes (12).

Massagué and his colleagues two opposite roles for astrocytes in brain metastasis in separate works: dispelling cancer cells entering the brain parenchyma (7,13) and promoting the progression of metastasis in the brain (8,14). The contact with astrocytes leads to up-regulated expression of multiple genes in the cancer cells (15), including several survival genes that are in charge of the increased resistance of cancer cells to cytotoxic drugs (12). Better therapeutic strategies need to be raised urgently.

Very recently, Lee *et al.* proposed that astrocytes protected cancer cells from paclitaxel. They demonstrated that the protection effect could be eliminated by an endothelin (ET) receptor antagonist that modulates the crosstalk between astrocytes and cancer cells (11). These findings have shed light on the combination of crosstalk modulators with anti-cancer drugs in brain metastasis treatment.

## Crosstalk that suppress the progression of metastasis

Specialized mechanisms are required for cancer cells to penetrate the blood-brain barrier (BBB), and most cancer cells that pass through the BBB will die (16). What kills most cancer cells that pass the BBB remains a question of biologic and clinical interest. Astrocytes have an integral role in preserving BBB properties, they support endothelial cells and imped cancer cells from traversing into the brain (17). Researchers noticed that only cells that grew on top of blood capillaries were able to survive, and each cell stick very closely to its vessel, just like a panda bear hugging a tree trunk. This hugging is indispensable, if a cancer cell detaches from its vessel, it will be killed by astrocytes nearby (7,16).

Astrocytes kill the infiltrated cancer cells by secreting a pro-apoptotic cytokine protein called Fas ligand (FasL) (18) that is highly expressed in reactive astrocytes in ischemia, Alzheimer's disease, encephalomyelitis, brain trauma, and multiple sclerosis (19). As early as 2008, it was demonstrated that plasmin cleaved FasL at Arg144, releasing sFasL as a diffusible cell death signal (20). Brain metastatic cells are susceptible to apoptosis if they are exposed to sFasL expressed by reactive astrocytes in the brain parenchyma (21).

Apart from converting FasL into a death signal for cancer cells, plasmin suppresses brain metastasis in another way. They inactivate L1CAM (22,23), a molecule expressed by metastatic cells for metastatic outgrowth and for spreading along brain capillaries. Both tissue plasminogen activator PA (tPA) and urokinase plasminogen activator (uPA) are implicated in the production of plasmin, they convert plasminogen into plasmin (24,25). Early studies found that lung cancer brain metastasis were infiltrated by reactive astrocytes, which are major sources of plasminogen activators (PAs) in ischemia and neurodegenerative injury (26). Abundant reactive astrocytes in the metastases lead to a resultant production of plasmin, which induce parent lung cancer cells to undergo apoptosis.

## Crosstalk that facilitate the progression of metastasis

Brain metastatic cells suffer Fas-dependent death (FADD) in the brain unless they are protected by anti-PA serpins (21). To prevent the generation of plasmin and its suppressive effects in metastasis, high levels of anti-PA serpins, are expressed by the survived cancer cells, including neuroserpin (NS) and serpin B2. The up-regulation of NS and serpin B2 in brain metastatic cells was confirmed at the protein level. Gene-expression data showed that the expression of SERPINI1 and SERPINB2, which encode NS and serpin B2 respectively, in the tumors was associated with brain relapse. In a word, anti-PA serpins provide a unified mechanism for the initiation of brain metastasis in lung cancer.

It seems that cancer cells that survive from the pro-survival signals such as anti-PA serpins will proliferate without a hitch (6), just like seeds find their appropriate soil. Chen *et al.* showed that it result from the establishment of gap junctions between cancer cells and reactive astrocytes (27). Researchers observed that cancer cells and astrocytes communicate with each other through gap junctions, and the communication activates pro-survival signals consistently. What's more, ions pass through gap junctions, and it was demonstrated as a potential mechanism of chemoresistance in lung cancer cells (9). It is shown that by means of protocadherin 7 (PCDH7), metastatic cells selectively form connexin 43 (Cx43) gap junctions with astrocytes, then cyclic GMP-AMP (cGAMP) is allowed to pass from cancer cells to astrocytes to activate STING, an inherent immune response pathway to cytosolic double-stranded DNA (dsDNA). The resulting production of tumour necrosis factor (TNF) and interferon-α (IFNα) in astrocytes supports outgrowth and drug-resistance in metastatic cells (27).

In addition to the direct interactions as gap junctions,

astrocytes can reprogram cancer cells through epigenetically regulators. It is shown that co-cultured cancer cells with murine astrocytes significantly reduced the expression of PTEN in cancer cells, and both human and murine tumor cells with normal PTEN expression lose the expression of PTEN after infiltration into the brain, but not to other organs. This, reversible, brain microenvironment dependent down-regulation of PTEN is regulated by microRNAs from astrocytes in the brain (28). This evidence provided a mechanism in which astrocytes induce a reversible loss of PTEN, an important tumor suppressor (29), in metastatic cancer cells.

## Crosstalks that enhance drug resistance of metastases

Drug resistance invariably limits the clinical efficacy of chemotherapy against brain metastasis. It has been generally acknowledged that drug resistance is mainly attributed to the impermeable structure of the BBB (30) and the expression of P-glycoprotein by cancer cells (31), but recently a novel mechanism illustrated that crosstalk between activated cancer cells and astrocytes led to the increased drug resistance (3,32).

Researchers found that co-culture of astrocytes with lung cancer cells led to up-regulation of survival genes in the cancer cells (24), and the up-regulation degree was directly correlated with increased resistance to all tested chemotherapeutic agents. The direct cell-to-cell interaction between astrocytes and cancer cells altered the pattern of gene expression in both cancer cells and astrocytes, including BCL2L1, GSTA5, and TWIST1. We further discovered that the up-regulation of the survival genes and consequent resistance are transient owing to the direct contact between the astrocytes and cancer cells through gap junctions (12).

However, the mechanisms how astrocyte stimulates the expression of survival genes in cancer cells remains unknown. Recent studies have proposed that activated ET signaling may protect cancer cells from the chemotherapeutic drugs induced cytotoxicity (33). As mentioned previously, reactive astrocytes are the main sources of metastases suppressors such as FasL and PAs. To date, accumulating evidence suggests that up-regulation of ET is also a distinctive feature of the reactive astrocytes that accompanies the pathologies of CNS diseases (34), including brain metastasis. As early as 1995, it was found that peritumoral astrocytes overexpress ET in most of the human brain metastasis cases (35). Kim et al. found that co-incubation of astrocytes with cancer cells with led to

up-regulated ET-1 expression in astrocytes and promoted the expression of ET receptors ($ET_AR$ and $ET_BR$) on the cancer cells significantly. They demonstrated that astrocytes protect cultured cancer cells from paclitaxel through an ET-dependent signaling that leads to up-regulated expression of anti-apoptotic proteins in cancer cells. What's more, the chemo-protective effect requires physical interaction between astrocytes and cancer cells can be abolished by antagonism of $ET_AR$ and $ET_BR$ signaling (33).

## New evidence

Very recently, Lee et al. reported that macitentan, a dual ET receptor antagonist, abolished astrocyte-induced protection of cancer cells to paclitaxel and increased overall survival of mice with experimental brain metastases (11). They found that both clinical and experimental lung cancer brain metastasis were infiltrated and surrounded by glial fibrillary acidic protein positive astrocytes, and $ET_AR$ and $ET_BR$ were heterogeneously expressed in the clinical brain metastasis samples. Dual antagonism of $ET_AR$ and $ET_BR$ signaling with macitentan in combination with paclitaxel significantly increases overall survival of mice with experimental brain metastases compared to the treatment of paclitaxel alone. Such findings reveals that although astrocytes up-regulate anti-apoptotic proteins in cancer cells through an ET-dependent signaling mechanism which provides protection of cancer cells, the chemo-protective effects can be eliminated by macitentan through blocking $ET_AR$ and $ET_BR$ signaling. The combination therapy of crosstalk modulators and anti-cancer drugs may provide a new strategy for the treatment of patients with brain metastases.

## Summary

Crosstalk between astrocytes and cancer cells demonstrate that astrocytes in the microenvironment affect the biologic behavior of tumor cells and strengthen the contention (*Figure 1*). To improve the therapeutic outcome, orchestration of the crosstalk in microenvironment is required in consideration of the complexity interaction between astrocytes and cancer cells. Pharmacological inhibition of gap junctions between cancer cells and astrocytes in mice was reported to suppress brain metastasis. There is no doubt that we must take tumor microenvironment into consideration when designing the therapy schedules. New evidence reported by Lee et al. applied the novel strategies into practice, drugs targeting the brain microenvironment and extrinsic mechanisms of
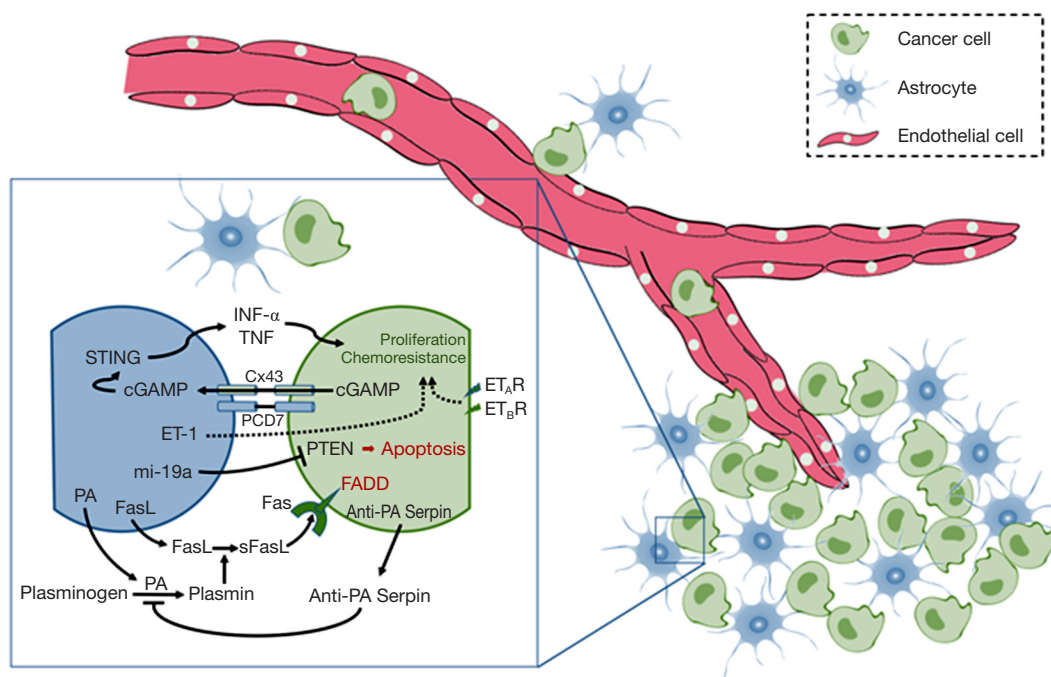
**Figure 1** Cartoon depicting bidirectional signaling between astrocytes and lung cancer cells (see text for details). IFN α, interferon-α; TNF, tumour necrosis factor; cGAMP, cyclic GMP-AMP; ET, endothelin; FADD, Fas-dependent death; FasL, Fas ligand; PA, plasminogen activator; Cx43, connexin 43; PCDH7, protocadherin 7.

resistance in cancer cells lead to beneficial effects in treating lung cancer brain metastasis. Therapies using cross-talk modulators may benefit the treatment of lung cancer brain metastasis in the future.

## Footnote

*Conflicts of Interest*: The authors have no conflicts of interest to declare.

*Comment on:* Lee HJ, Hanibuchi M, Kim SJ, *et al*. Treatment of experimental human breast cancer and lung cancer

brain metastases in mice by macitentan, a dual antagonist of endothelin receptors, combined with paclitaxel. Neuro Oncol 2016;18:486-96.

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