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COMMENTARY

Emperipolesis, entosis and beyond: Dance with fate

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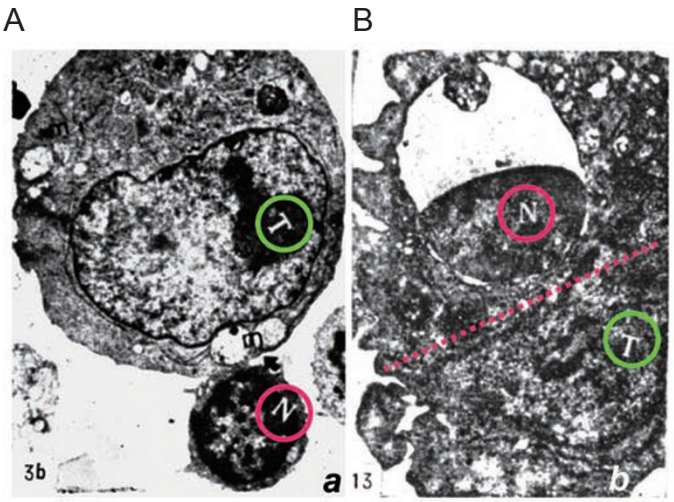
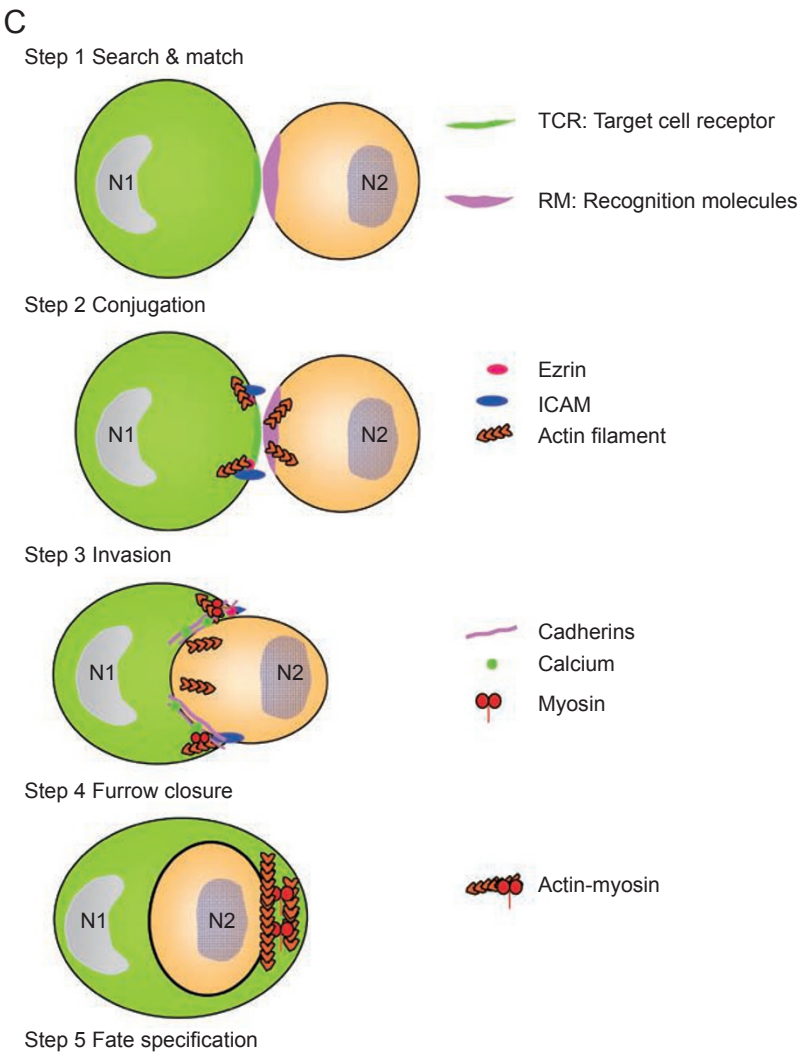
Study of cell-in-cell phenomena began with the desire to understand the complex phenotype, which stemmed from Lewis' observation of non-phagocytotic process of cell-eating-cell in 1925 [1]. Humble *et al*. coined the term "emperipolesis" (from the Greek, wandering round about within) in 1950s, to define the heterogeneous cell-in-cell phenomena when they studied biological interaction of lymphocytes with other cells [2]. Emperipolesis has since been found to be commonly enacted by lymphocytes in physiological and pathophysiological settings [3]. Since the term "emperipolesis" was annotated, a challenge has been set to understand how a cell inhabits in the other, what are their respective fates, and the biological relevance of co-habitation. Recently, Overholtzer *et al*. observed a homogeneous cell-in-cell phenomenon and named it as "entosis" (from the Greek, inside), an intercellular process that exhibits remarkable similarity to emperipolesis [4]. Further investigation of this entotic process revealed that it requires the formation of adherens junctions in the absence of integrin signaling, and force-driven invasion of one cell into another cell. In addition, the process requires the Rho-ROCK signaling pathway of the invading cell and myosin-based contractile force from the recipient cell. Surprisingly, some invading cells chose to run away via a transcytosis-like movement while a small percentage of invading cells underwent cell division within the host cells.

Interestingly, literature survey revealed that detailed cytological characterization of emperipolesis, very much similar to those observed in entosis [4], had been documented using natural killer cells and tumor cells in an extensive report published in the 1980s [5]. In pursuit of mechanisms underlying natural killer cell-mediated tumor cell death, Wang and Li had revealed that emperipolesis is a means to mediate natural killer cell-mediated tumor cell death (Figure 1A and 1B). This heterogeneous interaction requires membrane fluidity of target cell for its engagement with natural killer cells. They demonstrated disintegration of host tumor cell via a lysosome-mediated degradation pathway after the emperipolesis of natural killer cell at ultra-structural level. However, unexpectedly, they also discovered that some natural killer cells were disintegrated by tumor cells via a lysosome-mediated mechanism, similar to what was observed in entosis 20 years later. Occasionally, the invading natural killer cells underwent mitosis inside the host tumor cells after emperipolesis, similar to those observed in Overholtzer's report [4]. It was a remarkable observation to characterize the atypical and heterogeneous cell fates in the killer cell-tumor cell emperipolesis as most natural killer cells attack tumor cells via releasing cytolytic enzyme [6]. However, it has remained elusive how the internalized cells are delivered to or recognized by lysosomes during entosis and emperipolesis, and what determines the plasticity of internalized cells during emperipolesis.

Cell-cell adhesion is critical for the plasticity in tissues and organs. Adherens junctions are intercellular structures prominent in epithelia and neurons, which is composed of cadherins. Both emperipolesis and entosis require extracellular free calcium and adhesion molecules and actin-based cytoskeleton. Vasioukhin *et al*. reported that epithelial cells engage in a process of cadherin-mediated intercellular adhesion that utilizes calcium and involves actin polymerization [7], reminiscent of the initial phase of entotic and emperipoletic processes. Although it has long been recognized that calcium stimulates homotypic engagement of cadherins essential for intercellular adhesion [8], the precise mechanism for the calcium requirement is unclear. It seems likely that calcium and perhaps other divalent cations such as magnesium activate some key regulatory molecule(s) that in turn leads to actin polymerization at filopodia growth sites. Given the remodeling of cytoskeleton underlying the aforementioned processes, it would be of great interest to monitor whether intracellular calcium is mobilized and how. To systematically delineate emperipolesis and entosis, it would also be necessary to identify the key intercellular connection molecules es-





sential for the initial target survey and conjugation. Molecular characterization of protein-protein interactions underlying the aforementioned conjugation will be essential for illustrating the spatio-temporal dynamics of emperipolesis and entosis.

Molecular delineation of mechanistic processes underlying emperipolesis and entosis will require real-time visualization. The recent development of high-resolution fluorescence imaging protocol using two dyes preferentially labeling different fluid phases could directly provide a correlation between domain composition and local membrane curvature during the initial phase of search and conjugation [9]. Although cell protrusions have been described in different developmental processes, tissues, and organisms, their potential roles in cell signaling have been difficult to dissect. On one hand, most cellular processes are very fragile which prevents any biochemical preparation for reconstitution. On the other hand, those processes are very dynamic and mainly limited to live tissues. In these conditions it is technically challenging to define how the signaling is mediated via protrusion during conjugation. It is possible that signaling could occur through the release of free molecules, in a manner similar to synaptic neurotransmission, or shedding of vesicles as exosomes followed by entering the recipient cell. Alternatively, membrane-

Figure 1 Stepwise events underlying cell-in-cell process. **(A)** Electron micrograph illustrating the early stage when a natural killer cell (N) searches for a compatible tumor cell (T). **(B)** Electron micrograph illustrating the late stage when a natural killer cell (N) completely enters the tumor cell (T) prior to tumor cell disintegration. A dashed line illustrates the boundary between tumor cell and killer cell. Both **(A** and **B)** were taken from Wang and Li [5] with permission. **(C)** Hypothetic working model illustrates the stepwise events underlying cell-in-cell processes which appear common in emperipolesis and entosis.

tethered ligands on the protrusion could bind and activate receptors displayed on the surface of the receiving cell. The recent development of nano-scale imaging techniques, such as photo-activation localization microscopy [10], could enable us to visualize the molecular orchestra facilitating intercellular communications during the initiation connection in real-time emperipolesis and entosis.

Another exciting but challenging question is what determines the fate of invading cells versus that of recipient cells. In the case of entosis, the predominant fate of the internalized cells is lysosome-mediated degradation and non-apoptotic cell death [6]. It is unclear at present whether entosis is a means for cannibalism of the invading cell, which provides a survival advantage to the host cell through nutrient recycling during metabolic stress. In this case, suppression of entosis would be of significance in cancer treatment. Alternatively, entosis could represent a novel pathway to eliminate detached cancer cells, and thus its stimulation would offer a better outcome in clinical oncology. Given the fact that the progression of epithelial cell tumor is associated with the epithelial-mesenchymal transition, it will be interesting to determine whether entosis is aberrantly regulated in invasive cancer, or whether the aforementioned transition provides a selective advantage for cancer cells to escape from entosis. The preliminary observation that inhibition of Rho and ROCK activities blocks entosis in vitro and results in an increase in colony formation by mammary cell lines, argues for a role of entosis in tumor cell elimination [4]. Obviously, the ultimate question is how two cells of the same type duel for invasion and fate specification.

As for emperipolesis, the predominant fate of killer cells after invading tumor cells is lysosome-mediated degradation. One of the immediate interests is to elucidate the molecular pathways underlying cell fate determination upon emperipolesis and compare with those of entosis. One advantage in studying natural killer cell-mediated emperipolesis is the possibility of manipulating killer cell activity using cytokines such as IL-2. Helander *et al*. demonstrated that IL-2-activated target cell destruction by natural killer cells depends on the intercellular adhesion molecule ICAM-2 and is regulated by the polarized distribution of ezrin [11]. Interestingly, the spatiotemporal dynamics of ICAM-2 molecules are highly regulated in killer-sensitive cells in which ICAM-2 is concentrated into bud-like cellular projections by ezrin. The cytoskeletal-membrane linker protein ezrin is also localized in the polarized cellular structures such as uropods of target tumor cells [11]. Importantly, transfection of human ezrin into killer-resistant cells induces uropods formation, redistribution of ICAM-2, and sensitizes target cells to IL-2-activated killing. Thus, it would be of great interest to examine whether prior IL-2 stimulation would change the fate of natural killer cells in emperipolesis and whether its fate is related to ezrin plasticity [12].

While much of the excitement seen in the emperipoletic processes was mirrored in entosis, one obvious question is whether similar pathway operates in emperipolesis and entosis (Figure 1C). In addition, it would be of great interest to examine whether cell division of the invading cell is coupled to host cell cycle clock and how the two cycles coordinate or decouple in entosis and emperipolesis. In this case, entosis and emperipolesis may provide an excellent model system to study cell cycle regulation and cell-in-cell orchestration. Of medical importance, it would be necessary to determine whether entosis and emperipolesis are strategies for cancer progression and escaping from immunosurveillance. Certainly, molecular delineation of the processes of emperipolesis and entosis and the players and pathways that control them will be required to establish their functional relevance and specificities *in vivo*. Consolidation of protein-protein interaction networks combined with nano-scale imaging of molecular dynamics will enable us to illustrate the precise physiology and specificity underlying emperipolesis and entosis.

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