Molecular & Cellular Oncology 2:4, e1002707; October/November/December 2015; Published with license by Taylor & Francis Group, LLC

Cell-in-cell structures are involved in the competition between cells in human tumors

Qiang Sun[1,*], Hongyan Huang[2], and Michael Overholtzer[3,*]

[1]Laboratory of Cell Engineering; Institute of Biotechnology; Beijing, PR China; [2]Department of Oncology; Beijing Shijitan Hospital of Capital Medical University; Beijing, PR China; [3]Cell Biology Program; Memorial Sloan Kettering Cancer Center; New York, NY USA

Keywords: autophagy, cell competition, cell-in-cell, cell engulfment, cell death, entosis, entotic cell death, engulfment, cannibalism, phagocytosis

Abbreviations: CIC, cell-in-cell; KRAS, Kirsten rat sarcoma viral oncogene; c-Myc, cellular myc; E-cadherin, epithelial cadherin; P-cadherin, placental cadherin.

The engulfment of live cells may represent a mechanism of cell death. We reported that E-cadherin (epithelial cadherin) expression in human cancer cells favors the formation of cell-in-cell structures through the mechanism known as entosis, and that entosis contributes to a form of cellular competition in heterogeneous cancer cell populations.

Human tumors are heterogeneous and highly dynamic tissues that evolve from benign to malignant status. Although much has been learned over the last several decades about the genetics of tumor initiation and progression, the cellular mechanisms underlying clonal selection and tumor evolution remain poorly understood. In many cases cancers evolve by a long-term, stepwise process involving stages of oligoclonality,[1] in which multiple intratumoral cell clones compete in order to expand and invade normal tissue. How intratumoral cell clones interact with each other and with adjacent normal tissue is not well understood.

"Cell competition" was first reported in 1975 in the *Minute* mutant of *Drosophila melanogaster*, in which cells of heterozygous ribosome gene dosage, which are viable and able to form normal tissues, were found to be progressively eliminated when confronted with wild-type cells in mosaic tissues.[2] Further investigation led to the finding that the individual cells within developing organs undergo competition that contributes to tissue homeostasis, as only the fittest cells are selected for survival. Cell competition also occurs in mammalian tissues and may contribute to cancer initiation or progression, as wild-type cells could suppress tumorigenic outgrowth or, conversely, oncogene-expressing cells could induce the death of neighboring wild-type cells and invade normal tissue. In *Drosophila* and mammalian tissues, cells expressing certain oncogenes, for example cellular *Myc* (*c-Myc*), are indeed recognized as "fitter" and eliminate adjacent wild-type cells.[3,4] In this way, cell competition could promote tumor initiation by inducing so-called "field oncogenesis," whereby cells with activated oncogenic signaling replace wild-type cells in a phenotypically silent manner.[5] It is conceivable that intratumoral cell clones could also compete against each other, which could influence tumor evolution. Our recent findings demonstrate that entosis, a process by which winner tumor cells engulf and kill loser cells and benefit from their death, is one mechanism whereby cells within heterogeneous tumor cell populations might compete.[6-8]

Cell-in-cell (CIC) refers to the morphology of one or more viable cells present inside other cells, a phenomenon that has been documented in human tumors for many decades. One process leading to CIC formation is when epithelial cells or tumor cells engulf and kill their live neighbors by entosis.[9] We recently examined CIC formation in a panel of human breast tumor cells and found that the majority of cancer cell lines failed to form CIC structures, which correlated with a lack of expression of the epithelial cell-cell adhesion proteins E-cadherin (epithelial cadherin) or P-cadherin (placental cadherin). Entotic cell engulfment is known to require E- or P-cadherins, which mediate the engulfment process. The enforced expression of E- or P-cadherin induced CIC formation, suggesting a causal role of the loss of expression of these proteins in entosis resistance of human tumor cells. Expression of E- or P-cadherin also inhibited anchorage-independent growth, during which a high rate of entosis was observed, suggesting that entosis-mediated CIC formation and cell killing is a potential tumor suppressive pathway.[10]

*Correspondence to: Qiang Sun; Email: sunqiang@bmi.ac.cn; Michael Overholtzer; E-mail: overhom1@mskcc.org
Submitted: 12/17/2014; Revised: 12/18/2014; Accepted: 12/19/2014
http://dx.doi.org/10.1080/23723556.2014.1002707

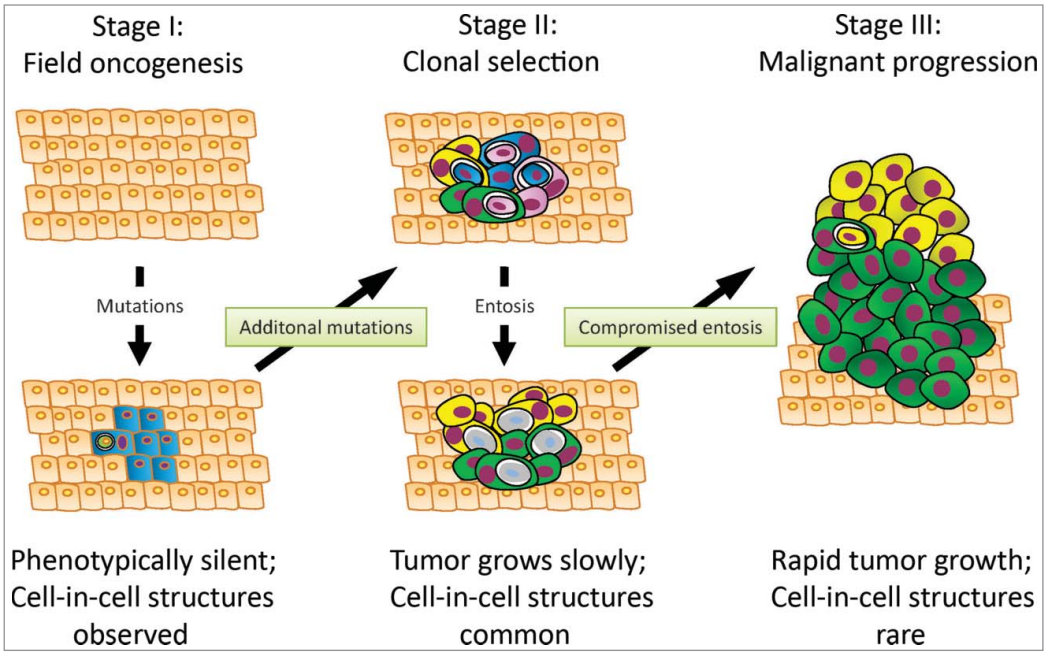

Figure 1. Hypothetical functional implications of entosis in oncogenesis and tumor progression. Entosis could conceivably facilitate field oncogenesis by replacing normal cells with mutant cells (stage I), or promote clonal selection by allowing winner cells to outcompete loser tumor cells (stage II). Some tumors may eventually reach an entosis-low state in which the cell population has become more homogeneous or epithelial-to-mesenchymal transition has inhibited this form of engulfment (stage III).

The idea that entosis might function as a mechanism of cell competition came from the observation that a number of tumor cells could consistently engulf normal or non-transformed epithelial cells when admixed.[6] Further investigation indicated that the identity of the winner, or engulfing, cells during entosis was actually genetically controlled. Tumor cells with certain oncogenic mutations such as *KRASV12* (Kirsten rat sarcoma viral oncogene homolog mutated glycine to valine at position 12), which has been implicated in *Drosophila* cell competition, could win over those with wild-type *KRAS* through entosis. This effect was mediated by Ras-related C3 botulinum toxin substrate 1 (RAC1)-dependent inhibition of Ras homolog gene family member A (RHOA) activity, which is a key regulator of cell stiffness through actomyosin contraction. Relative cell deformability was correlated with winner or loser cell identity during entosis, whereby the stiffer cells were usually internalized by their softer neighbors.[6] Thus, entosis allows cells harboring different genetic mutations to compete such that winner status is dictated by the relative physical properties of cells in a heterogeneous population. This mechanism of competition selects for cells with greater mechanical deformability, a cell property that is known to correlate with tumor progression and metastatic potential.[6]

Although entosis is now known to mediate a form of cell competition, a more detailed understanding of the effects of this kind of neighbor-induced cell death on tumor progression awaits further study. It is conceivable that entosis might have different effects on tumor progression depending on tumor stage (**Fig. 1**). For example, during the initiation of a primary tumor, entosis might participate in the spread of oncogene-expressing cells that replace cells from normal tissue by mediating the engulfment and death of surrounding wild-type cells. These early events would be predicted to be phenotypically silent. Once a tumor is formed, entosis might also mediate competition between different intratumoral cell clones, in which cells with mutations that reduce cell stiffness are predicted to dominate the tumor tissue at the expense of loser clones. Winner cells are also known to become polyploid by this mechanism[8] and to recover nutrients from the loser cells that they ingest,[7] suggesting that this form of competition could support tumor progression for winner clones by multiple mechanisms.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

The authors were supported by funding from the National Cancer Institute (CA154649, MO), the Benjamin Friedman Research Fund (MO), the National Basic Research Program of China (2015CB553704, QS), and the National Natural Science Foundation of China (81472588, QS).

References

1. Navin N, Kendall J, Troge J, Andrews P, Rodgers L, McIndoo J, Cook K, Stepansky A, Levy D, Esposito D., et al. Tumour evolution inferred by single-cell sequencing. Nature 2011; 472:90-4; PMID:21399628; http://dx.doi.org/10.1038/nature09807
2. Morata G, Ripoll P. Minutes: mutants of drosophila autonomously affecting cell division rate. Dev Biol 1975; 42:211-21; PMID:1116643; http://dx.doi.org/10.1016/0012-1606(75)90330-9
3. Moreno E, Basler K. dMyc transforms cells into supercompetitors. Cell 2004; 117:117-29; PMID:15066287; http://dx.doi.org/10.1016/S0092-8674(04)00262-4
4. Claveria C, Giovinazzo G, Sierra R, Torres M. Myc-driven endogenous cell competition in the early mammalian embryo. Nature 2013; 500:39-44; PMID:23842495; http://dx.doi.org/10.1038/nature12389
5. Slaughter DP, Southwick HW, Smejkal W. Field cancerization in oral stratified squamous epithelium; clinical implications of multicentric origin. Cancer 1953; 6:963-8; PMID:13094644; http://dx.doi.org/10.1002/1097-0142(195309)6:5%3c963::AID-CNCR282006-0515%3e3.0.CO;2-Q
6. Sun Q, Luo T, Ren Y, Florey O, Shirasawa S, Sasazuki T, Robinson DN, Overholtzer M. Competition between human cells by entosis. Cell research 2014; 24:1299-310; PMID:25342560; http://dx.doi.org/10.1038/cr.2014.138
7. Krajcovic M, Krishna S, Akkari L, Joyce JA, Overholtzer M. mTOR regulates phagosome and entotic vacuole fission. Mol Biol Cell 2013; 24:3736-45; PMID:24088573; http://dx.doi.org/10.1091/mbc.E13-07-0408
8. Krajcovic M, Johnson NB, Sun Q, Normand G, Hoover N, Yao E, Richardson AL, King RW, Cibas ES, Schnitt SJ., et al. A non-genetic route to aneuploidy in human cancers. Nat Cell Biol 2011; 13:324-30; PMID:21336303; http://dx.doi.org/10.1038/ncb2174
9. Overholtzer M, Mailleux AA, Mouneimne G, Normand G, Schnitt SJ, King RW, Cibas ES, Brugge JS. A nonapoptotic cell death process, entosis, that occurs by cell-in-cell invasion. Cell 2007; 131:966-79; PMID:18045538; http://dx.doi.org/10.1016/j.cell.2007.10.040
10. Sun Q, Cibas ES, Huang H, Hodgson L, Overholtzer M. Induction of entosis by epithelial cadherin expression. Cell Res 2014; 24:1288-98; PMID:25342558; http://dx.doi.org/10.1038/cr.2014.137