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Engulfing losers by winners in cancer: do cancer stem cells catch eat-me signals from noncancer stem cells?

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brain tumor; competition; cooperation; GBM; glioblastoma; glioma stem cells

“Better understanding of how interactions between cancer stem cells and noncancer stem cells contribute to the cancer stem cell phenotype will likely lead to the identification of potentially new molecular targets that will eventually establish strategies to treat this deadly disease.”

Each year, more than 1.6 million people in the USA are diagnosed with new cancers, and this number has been continuously increasing for decades. Most of the targeted therapeutics tested thus far have shown little or limited sustained survival benefit. The failure to achieve a cure may include the presence of self-renewing, highly tumorigenic cancer stem cells (CSCs) contributing to therapeutic resistance and invasion into normal tissues. Targeting CSCs is probably mandated to inhibit tumor growth and sensitize tumors to conventional therapies. In a tumor mass, however, CSCs are tightly associated with other cells including non-CSCs and stromal cells. Nonetheless, molecular mechanisms for the intercellular communication remain largely elusive. Recent studies in both mammalian and nonmammalian system have raised a possible novel concept that cellular competition between CSCs and non-CSCs may induce engulfment of non-CSCs by CSCs. The ultimate goals of CSC-directed medical research include the establishment of a new concept of inter cellular competition within tumors to better clarify the mechanism of tumor heterogeneity and targeted therapies for molecules responsible for a gain of resistance to current therapies. This concept, if proven true, would challenge the current research and clinical practice hurdles, thereby creating a firm path to development of a novel strategy to target the newly identified cellular alterations in cancers.

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Future perspective

Development of effective therapies for cancers is a challenging endeavor due to the highly aggressive and therapy-resistant nature of cancer [1,2]. Accumulated evidence suggests that some cancers, such as breast cancer and glioblastoma (GBM) retain steep cellular hierarchy with CSCs at the apex [1,3]. In these cancers, CSCs, as opposed to other cancer cells, are defined as those that can self-renew, give rise to a broad array of intratumor cell types and drive tumorigenesis. A tumor initiates from a single clone (a cell of origin), subsequently developing a large mass with heterogeneous cellular components. At the early tumor-initiating stage, microtumors are presumably surrounded with rich vasculature in the host tissue. In turn, rapid and aggressive proliferation of cancer cells creates an intratumoral microenvironment with extremely poor vascularity, which provides a restricted oxygen and glucose supply for individual tumor cells. Indeed, malignant tumors, such as GBM, display, in most cases, large necrotic lesions called central necrosis [4]. Interestingly, the perinecrotic hypoxic niche (microenvironment) is one of the intratumoral areas where CSCs predominantly reside [5]. Given that radio- and chemo-sensitivities are proportional to the intracellular oxygen level of tumor cells, it is important to molecularly and phenotypically delineate CSCs, in particular, in the perinecrotic niche, so that we can eventually establish targeted therapies for these CSCs. However, little is known regarding how limited nutrition is consumed by CSCs and non-CSCs in the perinecrotic area, and whether CSCs and non-CSCs cooperate or compete with each other for survival and growth.

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Drosophila is a useful model of cancer research, in particular, to study the complexity of intercellular communication *in situ*. The eye cancer models in *Drosophila* have demonstrated that various genetic and cellular mechanisms that are essential in normal organ homeostasis are hijacked in cancers to evade damage-induced cell death and paradoxically result in cancer expansion (e.g., the JAK–STAT pathway and Wingless pathway) [6,7]. Several intriguing theories have recently been proposed by utilizing several *Drosophila* cancer models. In particular, the concept of cellular competition [6,7] and fitness [8,9] has gained substantial attention. Unlike the homotypic situation, when cells of different genotypes share the same microenvironment, a struggle for existence between different clones occurs in malignant tumors *in situ*. Subsequently, one genotype (fitter or winner) outcompetes the other (loser) as a result of proliferative advantage. Cell competition is a well-known mechanism for intrinsic tissue homeostasis to maintain the integrity and normal development of organs. In the *Drosophila* cancer model in imaginal wing discs, less aggressive clones are eliminated by cell competition, which in turn accelerate malignant changes of the remaining more aggressive clones. To extend this concept, recent experimental evidence identified a more proactive cell competition process of cell assassination and corpse engulfment [7,10,11]. According to this idea, competent winner cells not only extrude loser cells from the microenvironment, but also actively engulf apoptotic debris, thereby winners overproliferate to compensate for cell loss and subsequently give rise to more aggressive cancers. Reasons for engulfment of loser cells by fitters remain to be determined; however, it is attractive to speculate that due to the limited

available nutrition in the tumor microenvironment, fitter clones efficiently absorb and utilize intracellular components derived from losers that are otherwise eliminated by immune cells. This hypothetical concept has not yet been extensively tested in human cancers. Nonetheless, this mechanism could potentially be clinically relevant, since the majority of cancer cells undergo apoptotic cell death after therapeutic insult. If any of these theories in the nonmammalian system is conserved in human cancers, novel molecular/cellular understanding may shed new lights on cancers' intercellular crosstalk, molecular mechanism for post-treatment cancer aggressiveness and subsequent reconstitution of recurrent cancers, and potential novel therapeutic targets. Obviously, in the developed countries, patients do not die due to *de novo* cancers, but by recurrence after therapeutic failure.

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Phagocytosis and clearance of dead or dying cells by immune cells is a well-characterized engulfment program in normal organs to maintain tissue homeostasis and remodeling. In turn, internalization of cells into nonimmune cells, termed ‘cell-in-cell’ structures, seems to not be a rare event and was originally reported as early as in 1864 – over a century ago [12–14]. Subsequent reports include Leyden's observation in 1904 that tumor cells occur inside other tumor cells. This phenomenon has been repeatedly reported to date. Intriguingly, the group led by Overholtzer and Brugge reported in 2007 that as many as one-quarter of breast cancer cells internalize sibling tumor cells in a suspension culture condition. Of note, engulfment may, at least, partly be distinct from immune cell-mediated phagocytosis for disposal in that unlike phagocytosis, engulfment is a process by which tumor cells take up not only dead cells, but also live cells for digestion.

Recently, Konishi's laboratory demonstrated that even embryonic stem (ES) cells actively engulf neighboring damaged hematopoietic progenitor cells. One difference in this study is that the data suggest active engulfment of apoptotic cells by ES cells, but it is not clear if they also engulf live cells [15]. Regarding the mechanism of action, the molecular and cellular process of ES cell engulfment seems to recapitulate the immune cell phagocytosis. Rho family GTPases and PI3K are required for cytoskeletal restructuring to form lamellipodia, where engulfment/phagocytosis takes place. In turn, apoptotic cells present ‘eat-me’ signals for phagocytotic cells in the phosphatidylserine-dependent manner. In response to these signals, recipient macrophages then undergo ‘cytoskeletal rearrangement’ via Rac1-mediated signals [16]. Presumably, engulfment by nonimmune cells (including cancer cells and stem cells) is an evolutionally conserved phenomenon from *Drosophila* to humans. Nonetheless, it remains largely unknown why and how some cancer cells incorporate other cells and the molecular mechanisms to provoke this phenomenon.

Aside from the engulfment theory, recent studies have also suggested two other mechanisms for how CSCs possess growth advantage over non-CSCs in limited nutrient microenvironment. Flavahan *et al.* reported that, under glucose-restricted conditions, CSCs competitively uptake glucose. According to this study, competitive uptake of glucose in a nutrition-restricted condition among heterogeneous tumor cell populations is not a random event [17]. The data from both *in vitro* cell cultures and *in vivo* mouse tumor models demonstrated that CSCs isolated from GBM tumor samples competitively incorporate

fluorescently labeled glucose, whereas non-CSCs fail to do so and subsequently undergo apoptotic cell death. In the end, surviving CSCs preferentially take over the majority of the cell populations and subsequently give rise to their derivatives (ironically including non-CSCs), when the available glucose is substantially limited.

As an alternative mechanism, a study by Mao *et al.* described that, after radiation treatment-induced DNA damage, CSCs gain a mesenchymal trait and activate the glycolysis and gluconeogenesis pathway. Transition of epithelial tumors to a mesenchymal phenotype is a well-known cellular transformation process in advancing aggressiveness, tumor cell motility, and metastasis in various types of human cancers [18]. Increased expression of the mesenchymal CSC markers (e.g., CD44) has been noticed in the perinecrotic glucose-restricted area – a CSC-enriched area in cancers [17,19]. In turn, the proneural (analogous to epithelial) CSC markers (e.g., Olig2) accumulate in the perivascular niche – the other CSC-enriched area in GBM. The glycolysis and gluconeogenesis pathway appears to be one of the most significantly differentially activated pathways in mesenchymal CSCs compared with proneural CSCs [20]. A shift of the CSC phenotype shift from proneural to mesenchymal cells is possibly one key process to advance tumor aggressiveness and therapy resistance.

In conclusion, CSCs represent a subpopulation of cells in single tumors, which are highly resistant to conventional therapies and exhibit tumor-initiating activity, thus making them critical drivers of cancers and attractive candidates as therapeutic targets. Intercellular communication between CSCs and the rest of the cells in tumors may play important roles in reestablishment of recurrent cancers, which may be paradoxically activated by current therapies. Better understanding of how interactions between CSCs and non-CSCs contribute to the CSC phenotype will likely lead to the identification of potentially new molecular targets that will eventually establish strategies to treat this deadly disease.

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Biography

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