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Cell Competition: Advances & mechanisms

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Abstract:

Cellular communication is an important process for animal development, which guides cell fate specification and the movement of cells within and between tissues. During growth, cell-cell communication plays a critical role in decisions that determine whether cells survive to contribute to the organism. Cell competition is one such remarkable phenomenon that is conserved from invertebrate to mammals, that causes the elimination of relatively less fit cells from tissues, helping to maintain overall tissue health. Cell competition is not only functional during development but it also replaces less fit cells in adult tissues. This suggests that the properties of individual cells are monitored and that variant clones of progenitor cells can be favored or eliminated accordingly. Progress has been made in recent years to understand the mechanisms of cell competition by several approaches but still much remains to be learned. Cell competition has been implicated in regenerative medicine, cancer and aging. It was assumed that molecular signals between cells are necessary and sufficient for cell competition. However, recent reports illustrate an interesting mechanism that has been previously speculated but never proven. In this review, we will discuss the process of cell competition and its' implications and mechanisms.

Keywords: Ribosomal Protein (Rp), Cell competition, Super competition, Homeostasis, Regenerative therapies, Field cancerization, Tumor

Discovery of cell competition

Cell competition was first discovered by Gines Morata and Pedro Ripoll in *Drosophila melanogaster* about forty years ago while studying the growth properties of '*Minute* mutations' that affect Ribosomal protein (Rp) genes (Garcia-Bellido et al., 1976; Morata and Ripoll, 1975). Cell competition is generally observed amongst cells having different growth properties while sharing the same developing compartment. The most fascinating feature of cell competition is that otherwise viable genotypes of cells can be eliminated depending on their interaction with neighbors. For example, many ribosomal protein (*Minute*) genes are essential and homozygous mutations are often lethal, but heterozygous *Minute* mutants generally exhibit a slow growth rate. *Minute* (*i.e. Rp*/+) cells are viable as whole animals of genotype *Rp/+* survive, but interestingly these *Rp*/+ cells are lost from genetic mosaics with wild type neighbors. A quantitative study showed that proliferation and survival rate of Rp/+ cells was reduced when wild type neighbors were nearby (Garcia-Bellido et al., 1973, 1976). Suggesting that, in developing tissues, cells can be favored or eliminated according to their relative fitness level with respect to their neighbors (Li and Baker, 2007; Tyler et al., 2007). Generally, cells that outcompete are known as 'winner' cells and those that get outcompeted are known as 'loser' cells. In last decade there are more developments in the field of cell competition in *Drosophila* as well mammals depicted in Table-1.

Cell competition was also observed in cells carrying different doses of the proto-oncogene '*myc*'. *myc* mutant cells are independently viable but get outcompeted when surrounded

by wild type cells (Moreno and Basler, 2004). Interestingly, cells with extra copies of *myc* can outcompete wild type cells from mosaics. In this situation, triplo-*myc* or tetraplo-*myc* cells act as 'supercompetitors' and outcompete otherwise viable wild type cells (de la Cova et al., 2004; Moreno and Basler, 2004). This reinforces the notion that cell competition is not due to internal defects but response to relative fitness amongst neighbors. The same genotype can be a winner or loser depending on neighbors, e.g. wild type cells (2 copies of *Myc*) win in competition with *myc* mutant cells but lose in competition with triplo-*myc* cells.

Supercompetition is also observed in cells that have mutations in tumor suppressor components of the Salvador-Warts-Hippo (SWH) pathway. SWH is a conserved kinase pathway that controls organ size in animals through the regulation of cell growth, proliferation and apoptosis (Pan, 2007). The SHW pathway components, when specifically mutated in *Rp/+* cells, could rescue *Rp/+* cells from cell competition when grown in mosaics with wild type cells (Tyler et al., 2007). Moreover, it was observed that cells with mutations in any of the SHW pathway components can outcompete wild type cells in mosaics, suggesting a super competitive ability like that observed with *Myc* (Chen et al., 2012; Hariharan and Bilder, 2006). Similar to *Myc*-induced super-competition, relative levels of SHW pathway components in neighboring cells can trigger cell competition. For example, proliferation of wild type cells is reduced, and they get outcompeted when grown in mosaics with cells over-expressing transcriptional coactivator protein Yki (which is homologous to mammalian YAP) (Chen et al., 2012). Yki is well known effectors of the Hippo signaling cascade that is required to activate expression of transcriptional targets that promote cell growth, cell proliferation, and prevent apoptosis and in turn tumorigenesis.

Another example is the remarkable-potential to undergo cell competition of tumor cells. Mutation of neoplastic tumor-suppressor genes like *scribble (scrib), discs large (dlg),* and lethal giant larvae *(lgl)* disrupt proteins that function as scaffolds at cell-cell junctions (Hariharan and Bilder, 2006). Loss of both copies of these genes leads to problems in the epithelial integrity of the cells leading to the development of neoplastic tumor (Hariharan and Bilder, 2006) and eventual death of the animal. Surprisingly, when such mutant cells co-exist in mosaic tissues alongside wild type cells, they get outcompeted and do not form tumors (Agrawal et al., 1995; Brumby and Richardson, 2003; Chen et al., 2012; Gateff, 1978; Woods and Bryant, 1991). The elimination of these cells is therefore competitive, since it does not occur in the homotypic environment. (Brumby and Richardson, 2003). All these data suggest that loss of epithelial integrity in cells can trigger elimination of those cells by cell competition that otherwise cause tumorigenesis.

There are several examples demonstrating that cell competition is not restricted to *Drosophila*. Evidences from mammalian systems have uncovered cell competition-like phenomena. When grown in mosaic with wild type cells, mouse cells, heterozygous for ribosomal protein gene (*RpL24/+*) show decreased proliferation and are outcompeted by wild type cells (Oliver et al., 2004). Differential *Myc* levels trigger cell competition in mouse development and also during cardiomyocyte replacement (Claveria et al., 2013; Villa del Campo et al., 2014). Cell competition has also been demonstrated in mammalian cell culture. MDCK cells mutant for the neoplastic tumor suppressors '*scribble'* or '*Mahjong*' (Norman et al., 2012; Tamori et al., 2010) or cells expressing Ras^{V12} (Hogan et al., 2009) survive in homotypic environment but get outcompeted when cultured with wild type cells.

Significance of Cell competition

The phenomenon of cell competition is conserved from flies to mammals, which suggests its evolutionary significance (Baker, 2011). When it was first discovered, cell competition was proposed to be a mechanism of growth control, providing homeostatic regulation, but it was later reported that cell death was a contributing factor for this homeostasis. So it indicates that cell competition might act in conjunction with other size regulatory mechanisms. Now cell competition is considered as a surveillance mechanism that selects for the 'fittest' cells during *Drosophila* and mouse development (Claveria et al., 2013). There is an intriguing relationship between cell competition and cancer progression (Baker and Li, 2008; Tamori and Deng, 2011).

Recent reports show that cell competition can function as both tumor suppressor and promoter. Epithelial cells harboring neoplastic tumor suppressor mutations (*Lgl, Dlg, Scrib*) lose polarity and causes massive tumor growth and metastasis when grown in isolation. However, when generated in the presence of wild type cells, these mutant cells are eliminated by cell competition and the integrity of the epithelium is maintained (Brumby and Richardson, 2003). In this case, cell competition acts as an anti-tumor surveillance mechanism to remove potentially cancerous cells as well as to achieve epithelial homeostasis. Conversely, if cells acquire mutations in hyperplastic tumor suppressor SHW pathway components (Tyler et al., 2007) or if they over-express the proto-oncogene *Myc* (de la Cova et al., 2004; Moreno and Basler, 2004), they do not lose epithelial integrity but continue to proliferate. Eventually, they eliminate neighboring wild type cells by super-competition (de la Cova et al., 2004; Moreno and Basler, 2004; Tyler et al., 2007). These tumor cells commandeer the intrinsic tumor suppressor mechanism of cells and expand by outcompeting wild type cells. These observations suggest that cell competition is a mechanism to select against pre-cancerous cells by eliminating them; unfortunately once tumor cells escape this cell competition they may expand by outcompeting neighboring cells. This phenomenon of super-competition is very similar to the process of field cancerization, a well-characterized process in tumor progression (Slaughter et al., 1953).

The important feature of cell competition is the replacement of one group of cells by another. Aside from the normal role of cell competition, it also has implications in regenerative medicine. For example, during liver repopulation in rat, host cells are taken over by transplanted fetal liver cells by a process very similar to cell competition (Oertel et al., 2006). A similar phenomenon has also been observed during cardiomyocyte replacement in adult mouse (Villa del Campo et al., 2014). A very recent work implied a role of cell competition-mediated elimination of less fit cells in the context of aging to extend life span of an organism (Merino et al., 2015).

Mechanisms of cell competition

The phenomenon of cell competition raises multiple questions about the survival of cells growing in a tissue. What is the signal for the relative fitness that makes one cell survive but causes the other to perish? What differences between cells trigger cell competition, and in what tissues? As cell competition was first observed in *Minute* mutants, it was thought that cell competition is caused by differential growth rates between the cells. However, it was later demonstrated that an increase of growth rate do not trigger cell competition when mutant cells are grown in mosaics with wild type cells (de la Cova et al., 2004).

There are many known signaling pathways that are implicated in cell competition, such as Bone morphogenetic protein (BMP) family member Decapentaplegic (Dpp), Jun-N-terminal kinase (JNK), Tumor Necrosis Factor (TNF), SHW, JAK/STAT, WNT, etc (Penzo-Mendez and Stanger, 2014). There are reports of genes that are involved in apoptotic corpse engulfment by winner cells after death of loser cells during competition (Claveria et al., 2013; Fullard et al., 2009; Li and Baker, 2007). Although there is also a report that contradicts this hypothesis (Lolo et al., 2012), and hence more work is needed to understand the involvement of engulfment in cell competition. Components of the innate immune system are also required for cell competition (Meyer et al., 2014). Microarray analysis of gene expression during cell competition led to the identification of several genes, such as *flower* (*fwe*), *sparc*, *ahuizotl*, etc. *flower* (a calcium channel) has different splice isoforms namely Fwe^{ubi}, Fwe^{loseA} and Fwe^{loseB}, and the expression of these different isoforms depend on the neighbor (Rhiner et al., 2010). During competition, winner cells recognize and induce Fwe^{lose} isoform in loser neighbor cells. Expression of Fwe^{lose} isoform in turn triggers the elimination of the cell. By contrast, the increased expression of Sparc (the homolog of the Sparc/Osteonectin protein family), a multifunctional, secreted glycoprotein, protects loser cells against death (Portela et al., 2010). In this case, cell competition could be a steady, reversible process, which blocks removal of cells that have inconsequential short-term fluctuations in gene expression. *ahuizotl* (*azot*) (an EF-hand-containing cytoplasmic protein) expression is specific to suboptimal but otherwise morphologically viable cells. –*azot* mutants show increased morphological malformations, susceptibility to accidental mutations, and accelerated tissue degeneration. Azot works with the above-mentioned molecular players and acts as a fitness checkpoint to remove less efficient cells (Merino et al., 2015). This was the first report to show that cell competition is beneficial for tissue health and has positive effects on lifespan and, in turn, aging.

In addition to molecular signals that were summarized in the previous paragraph, there are new reports uncovering novel mechanism for cell competition. It has been demonstrated that mechanical forces are responsible for the elimination of cells during cell competition, a process termed as 'mechanical cell competition' (Levayer et al., 2016; Wagstaff et al., 2016). MDCK cells that lose '*scribble*' ($scrib^{KD}$) get outcompeted when grown with wild type neighbors (Norman et al., 2012). These $scrib^{KD}$ cells are hypersensitive to compaction and display elevated tumor suppressor protein p53 (Wagstaff et al., 2016). Compaction of $scrib^{KD}$ cells causes activation of the Rho-associated kinase (ROCK) that in turn activates p38 leading to further p53 elevation. This mechano-transduction cascade leads to death of $scrib^{KD}$ cells. Elevation of p53 is necessary and sufficient to induce a mechanical loser status due to compaction (add Wagstaff et al., 2016). p53 is a general sensor of cell stress, so it is possibility that mechanical cell competition may be widespread among the damaged cells. Since p53 is a tumor suppressor gene commonly mutated in cancer, it is possibility that neoplastic cells can circumvent mechanical cell competition when lose p53. Cancer cells expressing oncogene Ras can compress neighboring wild type cells and can eliminate cells up to several cell diameters away from the clones (Levayer et al., 2016). This mechanical super-competition could be involved in tumor growth as well.

Despite all the progress in the field, the exact mechanism of cell competition is not clear yet. The phenomenon of cell competition is

conserved through evolution that proves its significance (or "Conservation through evolution proves the biological significance of cell competition). Moreover, cell competition has implications on processes such as cancer, regenerative medicine, and aging. There are many opportunities to associate the known molecular pathways of cell competition and, most probably, to uncover new ones in order to define the adaptive roles of cell competition. Future research in the field will shed some light on such functions of cell competition.

Table: 1 Summary of different cell competition scenario studied.

Winner	Loser	Organism	Downstream effectors
Wild type cells	*Minute* mutants (*Rp/+*)	Drosophila	JNK,p53,Azot,NF-κB,Sparc
Wild type cells	Neoplastic tumor cells (*scrib,lgl,dlg*)	Drosophila MDCK cells	JNK,p53
Transplanted fetal liver cells	Older liver cells	Rat	Unknown
Higher level of Myc	Wild type cells	Drosophila, Mouse	JNK,p53,Azot,NF-κB,Sparc
WNT signaling Hyperactive cells	Wild type cells	Drosophila	JNK,Azot
Mutants of Hippo (SWH) pathway	Wild type cells	Drosophila	Unknown

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