Cell Competition Boosts Clonal Evolution and Hypoxic Selection in Cancer

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The comparison of fitness between cells leads to the elimination of less competent cells in the presence of more competent neighbors via cell competition (CC). This phenomenon has been linked with several cancer-related genes and thus may play an important role in cancer. Various processes are involved in the regulation of tumor initiation and growth, including tumor hypoxia, clonal stem cell selection, and immune cell response, all of which have been recently shown to have a potential connection with the mechanisms involved in CC. This review aims to unravel the relation between these processes and competitive cell interactions and how this affects disease progression.

**CC, Associated Processes, and Cancer**

CC is a process by which measurements of relative fitness in neighboring cells allow communities to eliminate defective or damaged cells (called ‘losers’) to favor the proliferation and growth of competent cells (‘winners’) [1,2]. Several CC mechanisms exist, notably the comparison of ‘fitness fingerprints’ [3]. These aid in maintaining homeostasis, and defects in CC mechanisms are implicated in ageing and carcinogenesis [4,5]. Numerous processes influence carcinogenesis: (i) hypoxia promotes aggressiveness and resistance to therapy [6,7]; (ii) clonal selection of stem cells contributes to tumor development and heterogeneity [8,9]; and (iii) immune cell responses select and affect the outcome of cancer and cancer cells [10,11]. Tumor cells start growing in a hostile environment and depend on CC mechanisms to acquire a competitive state, which allows them to overcome the continuous repression of neighbor cells [12,13]. This seems to be conserved in mammals [3].

**Flower-Based CC and Its Role in Cancer**

In humans, C9ORF7 encodes for four splicing variants of the transmembrane protein Flower (hFWE1–4) [3]. Recent data showed that cells expressing hFWE isoforms under monoculture conditions are not eliminated by apoptosis. However, co-culture studies revealed that cells expressing hFWE2 or hFWE4 proliferate and trigger caspase-dependent apoptosis of cells expressing hFWE1 or hFWE3 [3]. Thus, hFWE2 and hFWE4 are considered hFWE\textsuperscript{Win} isoforms, whereas hFWE1 and hFWE3 are considered hFWE\textsuperscript{Lose} isoforms. These data suggested that Flower isoforms act as fitness fingerprints in CC events based on the comparison of the isoforms expressed by cells [3].

Analyses of human tumor samples revealed that tumor cells express predominantly hFWE\textsuperscript{Win} isoforms, whereas the adjacent stromal cells express hFWE\textsuperscript{Lose}. In normal tissue, all hFWE isoforms are poorly expressed [3]. Further analyses of the tumor microenvironment (TME) showed that the expression of hFWE\textsuperscript{Lose} isoforms as well as the expression of genes involved in apoptosis increases as stromal tissue approach the edge of tumor tissue [3]. Overall, these data suggested that tumors can outcompete their adjacent cells through Flower-mediated CC.
Further work in mice showed that tumor cells overexpressing human Fwe\textsuperscript{Win} form larger tumors and develop more metastases in mice with a Fwe\textsuperscript{Lose} background than in mice with a Fwe\textsuperscript{KO} background [3]. Additionally, the expression of Fwe\textsuperscript{Lose} increases in Fwe\textsuperscript{WT} mice harboring Fwe\textsuperscript{Win} tumors. Therefore, the interaction between the tumor and the TME may contribute to tumor progression, as Fwe\textsuperscript{Win} tumors promote the non-autonomous expression of Fwe\textsuperscript{Lose} in stromal tissue [3].

The expression levels of hFWE\textsuperscript{Win} influence the tumorigenic potential of cells, as metastatic cells with higher expression of hFWE\textsuperscript{Win} develop larger tumors than cells with lower expression of hFWE\textsuperscript{Win} [3]. Moreover, the tumorigenic potential of cells expressing hFWE\textsuperscript{Win} decreased on its knockdown (KD), as these cells formed smaller tumors with less metastatic potential [3]. These findings were further enhanced when hFWE\textsuperscript{Win} KD was combined with chemotherapy, suggesting that combining hFWE target therapy with standard-of-care therapy could decrease tumorigenicity by impairing growth and metastatic potential in tumors [3].

Multiple CC Pathways in Regulation of Cancer

Tissue homeostasis is under a strict balance between proliferation of competent cells and elimination of cells harboring defects, such as mutations that could result in cancer development [14,15]. To maintain this balance, cells use defense mechanisms that allow them to identify and eliminate these defective cells. One of such mechanisms is the epithelial defense against cancer (EDAC), which results in the apical extrusion (see Glossary) of potentially oncogenic cells from the epithelial layer [14]. EDAC is a CC mechanism in which normal cells surround and eliminate, in a nonautonomous fashion, cells expressing oncoproteins such as RasV12, Erb, Src, or constitutively active forms of Cdc42 [14]. The surrounding normal cells influence cytoskeletal rearrangements that result in the accumulation of cytoskeletal proteins at the boundary, generating contractile forces and metabolic changes on transformed cells, which facilitates their apical elimination from the epithelial layer [14].

Cells harboring mutations bypass tissue surveillance systems such as EDAC, hijack CC mechanisms, and eliminate normal cells causing diseases such as cancer [14]. One of the most-studied proteins associated with CC events, which is known to develop the supercompetitor phenotype, is MYC [16]. MYC is frequently upregulated in many cancers and drives tumorigenesis by inducing metabolic shift and promoting cell growth and proliferation [17]. In human cancers, the overexpression of MYC in tumor cells causes the nonautonomous apoptosis of adjacent stromal cells by activation of the caspase pathway [18]. CC assays in Drosophila and in human cancer cell lines have shown that cells can sense different expression and activation levels of MYC as a fitness marker. When cells with different levels of MYC are cultured separately, their proliferation and apoptotic rates are similar [17]. However, when these cells are co-cultured, those with higher levels of MYC have increased proliferation rates and induce the elimination by apoptosis through caspase activation of those expressing lower levels of this protein [17,18].

Intriguingly, MYC-mediated CC mechanisms have been found to be regulated by the Hippo pathway, as a consequence of the loss of cell polarity. This has been observed in Drosophila and humans [18]. Deregulation of HUGL-1 (homolog of Drosophila lgl) causes loss of polarity and promotes the nuclear translocation of YAP (homolog of Drosophila Yki), leading to MYC overexpression [18]. YAP is highly expressed in various cancer types and its activation can further facilitate tumorigenesis by promoting cell proliferation, migration, survival, and chemoresistance [19,20] and by modulating the immune system [19]. Recently, a study using glioma cells in mouse models demonstrated that differential YAP expression in tumor cells triggers competition events thus contributing to tumor progression [19]. In co-culture conditions, cells expressing...
higher levels of YAP have enhanced growth and cause the elimination by apoptosis of cells expressing lower levels of this protein [19]. Moreover, RNA-seq data from cells expressing higher and lower YAP levels cultured in a homogeneous or heterogeneous environment showed that a set of tumorigenesis-related genes is induced in cells on competition [19].

Mutations in TP53 are commonly found in cancer cells and may result in abrogation of p53 tumor suppressive functions, such as cell-cycle arrest and apoptosis [21]. This allows the accumulation of oncogenic mutations and favors resistance to genomic instability and other stress conditions, such as starvation and hypoxia [21]. Several studies have revealed the roles of p53 in CC [22,23]. As an example, increased p53 levels in Bmpr1a−/− cells inhibit mTOR signaling and decrease their metabolic activity, thus causing their elimination by apoptosis when co-cultured with wild-type (WT) cells [22]. Additionally, mechanical compression can induce p53 elevation in less-fit cells and cause their elimination by apoptosis [23]. Furthermore, p53 expression is higher in Myc supercompetent cells and is required to enhance metabolic reprogramming and sustain cell proliferation [24]. Thus, the accumulation of mutant forms of p53 in cancer cells could simultaneously confer resistance to apoptosis and support cancer cell proliferation through Myc activity.

In this review, we prepare a revised summary of CC genes that are involved in the regulation of cancer, in human and mouse models (Table 1) [3,5,17,22,23,25–42].

Table 1. List of Genes Involved in CC Mechanisms That Play Important Roles in the Regulation of Cancer

<table>
<thead>
<tr>
<th>Gene</th>
<th>Refs</th>
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<tbody>
<tr>
<td>BMP6</td>
<td>[35]</td>
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<tr>
<td>CACFD1</td>
<td>[3]</td>
</tr>
<tr>
<td>COL17A1</td>
<td>[3]</td>
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<tr>
<td>EGFR</td>
<td>[36]</td>
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<tr>
<td>HRAS</td>
<td>[32]</td>
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<tr>
<td>JAK/STAT</td>
<td>[38]</td>
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<tr>
<td>KRAS</td>
<td>[41]</td>
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<tr>
<td>MAPK14</td>
<td>[23]</td>
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<tr>
<td>MAPK8 (JNK)</td>
<td>[39]</td>
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<tr>
<td>MAPK9</td>
<td>[28]</td>
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<tr>
<td>mTOR</td>
<td>[22]</td>
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<tr>
<td>MYC</td>
<td>[17]</td>
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<tr>
<td>MYCN</td>
<td>[25]</td>
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<tr>
<td>NRAS</td>
<td>[33]</td>
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<tr>
<td>PPM1D</td>
<td>[29]</td>
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<tr>
<td>SCRIB</td>
<td>[37]</td>
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<tr>
<td>SPARC</td>
<td>[28]</td>
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<tr>
<td>SRC</td>
<td>[34]</td>
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<tr>
<td>TAZ</td>
<td>[42]</td>
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<tr>
<td>TEAD1</td>
<td>[40]</td>
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<tr>
<td>TP53</td>
<td>[31]</td>
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<tr>
<td>WNT1</td>
<td>[27]</td>
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<tr>
<td>YAP1</td>
<td>[30]</td>
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</table>

*References are the original work on each gene.

Hypoxia and Its Influence on Cancer

Hypoxia is characterized by deficient oxygen levels leading to cellular changes largely driven by hypoxia inducible factor (HIF) signaling [43]. HIFs are a family of transcription factors – HIF-1, HIF-2, and HIF-3 – comprising an alpha subunit that is stabilized on hypoxia and a constitutively expressed beta subunit (ARNT) [6]. HIFs induce changes in the cellular transcriptome controlling metabolism, glucose uptake, proliferation, differentiation, and survival as well as angiogenesis and erythropoiesis [6]. Although it has been demonstrated that HIF-1α may play opposite roles in tumor progression [44], studies show that, in a hypoxic microenvironment, activated HIF-1α may promote epithelial-to-mesenchymal transition (EMT), which in turn promotes invasion, a cancer stem cell (CSC)-like phenotype, and resistance to chemotherapy [6]. HIF-1α can also promote cancer migration by regulating the expression of enzymes that polymerize thus regulate the alignment of collagen fibers and the activity of integrins [45]. Last, hypoxia promotes endothelial cell contraction, which makes vessels leaky and aids the passage of metastatic cancer cells through blood and lymphatic vessels [46]. It has been observed that the HIF-1α protein is overexpressed in several solid malignancies, including breast, colon, gastric, lung, skin, ovarian, pancreatic, and prostate, compared with their respective normal tissues [6]. Furthermore, in response to hypoxia HIF-1 can activate the multidrug resistance 1 (MDR1) gene, thus promoting chemoresistance [45]. Taking these findings together, hypoxia promotes tumor growth and metastasis (Figure 1A).

Hypoxia Regulates the Expression of CC Genes

Some CC genes in Table 1 have been shown to play a role in oncogenesis, cancer growth, or metastasis and to exhibit
(A) High O₂
Low O₂

Normoxic cells
Hypoxic cells
Apoptotic cells

(B) Mutagenic insult
WT p53
MUT p53
Mutant p53 promotes a winner phenotype

Malignant neoplasm

Hypoxic region
HIF1α

WT p53
p53–HIF-1α complex
p53–HIF-1α complex promotes a winner phenotype

(C) H&E
HIF-1α IHC

Cell fitness
Winner triggers:
MYC, WNT, DPP, JAK, Hippo, Scribble

Downstream effectors:
NJK, p53, Azot, NF-κB, Sparc

(See figure legend at the bottom of the next page.)
differences in their expression profiles under hypoxic conditions, thus contributing to tumor aggressiveness.

Some studies have uncovered a relationship between hypoxia and the Wnt/β-catenin pathway [47–49]. A study found that WNT11, which is highly expressed in several cancers and implicated in proliferation, migration, and invasion, is induced by hypoxia in various cell types [47]. Under hypoxic conditions, HIF-1α augments WNT11 expression, thus promoting the migration and invasion of tumor cells through the activation of matrix metalloproteinase (MMP)-2 and MMP-9 [47]. Additionally, in vivo experiments showed that WNT11 is mainly expressed in hypoxic areas and its KD reduces tumor growth in mice, which suggests that WNT11 may increase the metastatic potential of tumor cells under hypoxia [47].

Bone morphogenetic proteins (BMPs) have been previously associated with CC. In mammals, pluripotent cells with decreased BMP signaling are eliminated in the presence of WT cells [22]. Several studies have connected increases in BMP levels with patient outcomes in many cancers, including hepatocellular carcinoma (HCC) [50]. It was shown that hypoxia induces BMP4 expression in HCC cells and that two Ets-1-binding-sites in the BMP4 promoter have increased activity under hypoxic conditions [50]. Further, transfection of the dominant-negative HIF-1α isoform abrogates BMP4 expression [50]. This hypoxia-regulated expression of BMP4 supports the idea that CC genes may play a role in the increased tumor aggressiveness caused by hypoxia.

A study demonstrated that hypoxia can activate YAP by promoting its nuclear localization in HCC cells [20]. Under this condition, YAP binds to HIF-1α in the HCC cell nucleus, thus sustaining HIF-1α stabilization, which promotes cell glycolysis. Moreover, it was shown that silencing YAP inhibits HCC cell glycolysis in hypoxic conditions and reduces their invasion and migration [20]. This example suggests that YAP is involved in promoting cancer cell glycolysis in hypoxia thereby contributing to tumor cells’ aggressiveness.

HIF-1α and HIF-2α demonstrate disparate roles in their regulation of c-Myc expression and function, dependent not on the level of hypoxia but instead on its duration [51]. Acute hypoxia (2 h) upregulated c-Myc mRNA and protein expression via HIF-1α activity, driving chemoresistance of colorectal cancer cells and thus enhancing tumor aggressiveness. By contrast, HIF-2α controlled c-Myc expression during chronic hypoxia (over 8 h), leading to chemosensitivity [51]. HIF-1α upregulates p53 expression by binding to HIF-1α response elements (HREs) and interacts with p53, despite its mutational status, to form a HIF-1α–p53 complex [6]. In this complex, p53 adopts a mutant-like conformation, which indicates that, under hypoxic conditions, cells adopt a phenotype reminiscent of a mutant p53 genotype regardless of whether they harbor a mutation in TP53. Notably, hypoxia also selects for apoptosis-resistant cells [52]. Taking these findings together, hypoxic cells can outcompete normoxic WT TP53 cells through CC, and this p53-dependent mechanism then promotes cancer aggressiveness (Figure 1B). Thus, we

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**Figure 1. Cell Competition-Dependent Mechanisms That Promote Cancer Aggressiveness.** (A) Representation of a tumor region where tumor cell proliferation and/or vessel dysfunction has created a site of hypoxia distal from accessible oxygen diffusion. Other tumor regions remain with normal oxygen levels (normoxia). Hypoxia activates pathways in some hypoxic cells that generate a fitness winner phenotype, while neighboring cells in the hypoxic region display a fitness loser phenotype and undergo apoptosis. (B) Cell competition selects for hypoxic cells and MUT p53 cells. Under hypoxic conditions, p53 complexes with hypoxia inducible factor 1 alpha (HIF-1α) and acquires a mutant-like conformation independent of its genetic sequence. Hypoxic cells and cells harboring a p53 mutation (MUT p53) outcompete their normoxic wild-type (WT) counterparts, leading to neoplastic growth. (C) Human high-grade serous ovarian carcinoma sample stained with hematoxylin and eosin (H&E) (left) and with anti-HIF-1α (right). The image shows hypoxic tumor regions with predominant HIF-1α stabilization (nuclear staining; orange arrowheads) and normoxic tumor regions (blue arrowheads). Hypoxia has been reported to trigger cell fitness pathways including MYC, WNT, DPP, JAK, Hippo, and Scribble and their downstream effectors JNK, p53, Azot, NF-κB, and Sparc.

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propose a model to depict the relationship between hypoxic and normoxic zones in cancer and the role of CC genes and HIF signaling (Figure 1C). To further support our model, we performed a secondary analysis of publicly available RNA-seq data to highlight the relationship between CC gene expression and hypoxia (Box 1).

Role of CC in Selection of Therapy-Resistant Stem Cell Populations

Stem cells exist in niches adapted for their growth and that provide signals for their maintenance [53]. Therefore, stem cells compete continuously with their neighbors for niche occupancy [54]. CC can function as a tumor suppressor system, by eradicating, as an example, cells with apicobasal polarity mutations from stem cell niches [4]. However, stem cells with malignant propensities can also use CC mechanisms to outcompete normal stem cells and colonize these niches [4]. Stem cell populations are maintained by asymmetric divisions and can be lost or replaced stochastically to maintain homeostasis, in a process termed neutral competition [54,55]. Another type of stem CC, termed non-neutral competition, occurs between two unequal stem cell populations. The development of mutations in a fraction of stem cells in a niche can lead to the selection/elimination of stem cells that have a competitive advantage/disadvantage over their non-mutant neighbors [54]. Non-neutral competition can arise in CSCs as a response to different therapies. With mutations that help CSCs evade various therapies, the population becomes enriched with resistant mutant CSCs due to selective pressure.

The clonal evolution (CE) model and the CSC model are two independent models of cancer development, but recent studies highlight the intersections between these models [8,56]. The CE model states that cancer development is an evolutionary process, with the accumulation of genetic and epigenetic alterations resulting in complex heterogeneity [57]. The CSC model hypothesizes that cancers are initiated through initiator cells and states that cancers emerge from a restricted fraction of CSCs [58]. Some investigators believe these two models are complementary and should be combined as they highlight different aspects of the same cancer [8,59], and it has been shown that CE occurs in CSCs [8] and that CSCs can thus represent a heterogeneous target, with multiple subpopulations with various therapy sensitivities coexisting, competing, and evolving in the same tumor (Figure 2A). Further accumulation of mutations in CSCs will result in differentially behaving populations. A new mutation could lead to the acquisition of migratory ability, thereby separating ‘stationary CSCs’ from ‘migrating CSCs’, which can form metastases [8]. This heterogeneity and CE contribute to CSC competition leading to the complexities of cancer detection, targeting, and prognosis. The instructive value of the CSC model relies on two premises: first, not all cancer cells are CSCs; and second, CSCs can be distinguished from non-CSCs and CC favors the survival of CSCs. Consequently, CSCs can survive stressful conditions such as the presence of chemotherapeutic agents, hypoxia [60], or radiotherapy [61].

CC Pathways Mediate the Relation between Cancer and Immune Cells

The immune system is balanced between pro- and anti-inflammatory states and a one-way deviation can lead to autoimmunity or cancer, respectively [62]. This balance requires constant replenishment and trophic support for various cell subsets to prevent pathology. For example, depletion of regulatory T cells (Treg) abrogates peripheral tolerance and promotes autoimmunity [63]. The way in which CC regulates interactions between immune cells is not well understood. From the bone marrow to peripheral tissues, immune cells must respond to various environmental cues to function and mature properly. The tissue microenvironment significantly affects competition by providing different factors. One study highlights the importance of CC in the maintenance of immune function and development to prevent incipient immune cell neoplasia by subjecting immune cell populations to competitive pressures in the thymus [64]. Additionally, pathological niches such as TME have a unique set of rules that influence the interaction between immune cells.
Box 1. CC-Dependent Mechanisms That Promote Cancer Aggressiveness

We have proposed a model depicting the relationship between hypoxic zones of cancer, CC, and the role of CC genes in manifesting HIF-regulated signaling, eventually promoting cancer growth and aggressiveness (Figure 1C in the main text). To identify the relation between the genes that control CC mechanisms (Table 1) and hypoxia in solid tumors, we performed a secondary analysis of CC genes from HCT-116 cultured under hypoxia and normoxia (Figure IA). The results show that many CC genes are differentially expressed under hypoxic conditions compared with normoxic (Figure 1A,B). Using a combined list of the top-200 upregulated genes on hypoxia and our list of CC genes, we performed gene/protein co-occurrence/interaction mapping using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://string-db.org). STRING analysis shows significant correlations between CC genes and genes that are upregulated under hypoxia (Figure 1C).

Figure 1. RNA-Seq Reveals Differential Expression of Cell Competition (CC) Genes Enriched under Hypoxic Conditions. (A) Heatmap of the expression of CC genes (Table 1) from publicly available RNA-seq data (GSE101526). Expression of CC genes was analyzed in HCT-116 cultured under hypoxia or normoxia. The FPKM values were used to calculate differential expression of genes. The heatmap shows averaged expression of the CC genes in the groups using z-score normalization. Differential expression analysis of CC genes was conducted using DESeq2. (B) Bar plot of ten CC genes from the heatmap that were significantly differentially expressed (P < 0.05, Student’s two-sided t-test). (C) Using a combined list of the top-200 upregulated genes on hypoxia and our list of CC genes, we performed gene/protein co-occurrence/interaction mapping using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://string-db.org). We used only the correlations between genes from experiments and databases’ gene interaction catalogs of the STRING software. With those, we excluded random interactions, caused by co-occurrence in the paper texts, as well as other forms of data mining, and kept only the genes for which interactions were properly filed. We utilized the highest stringency (0.9) on correlation analysis instead of the default medium stringency (0.4). The genes/proteins related to the GO term ‘Response to hypoxia’ are marked with blue. The genes/proteins associated with the KEGG term ‘hsa05200 Pathways in cancer’ are marked in red. Both terms were significantly enriched in the input set of genes (FDR = 1.16e-14 and FDR = 1.66e-15, respectively), which excludes their appearance as a random event. Data analysis and visualizations were performed in R, using DESeq2, gplots, viridis, RColorBrewer, dplyr, ggplot2, readr, plyr, tidyverse, readxl, ggplot, EnstDb.Hsapiens.v96, and ggridges.

Trends in Cell Biology

CC in the Bone Marrow: A Tumor Suppressor Mechanism for Hematological Cancers

Immune cell precursors develop from hematopoietic stem cells (HSCs) replenished by the bone marrow. Studies in HSCs have shown that p53 mediates CC in a manner independent of its DNA-damage response [31,65], as relative (not absolute) levels of p53 lead to growth arrest by
neighbors with lower p53 levels rather than apoptosis. This mechanism minimizes the risk of development of hematological neoplasia by permitting clonal expansion of HSCs with low p53 levels and, presumably, low mutational burden or stressful insult.

Mutations in p53 can cause myelodysplastic syndrome (MDS) and acute myeloid leukemia [66]. Mutant-p53 HSCs can trick their neighbors into thinking they are fitter. How cells compare p53 levels remains a mystery. Soluble factors, cell–cell interactions and minerals could facilitate the communication of p53 levels between HSCs. Moreover, mutations in p53 have been shown to affect the secretome of cells to favor tumorigenesis, metastases, and immunoregulation [67]. HSCs exist in a high-Ca²⁺ environment and must limit their intracellular Ca²⁺ to maintain proliferative capability [68]. The known fitness markers SPARC and Flower both serve as Ca²⁺ channels and may consequently facilitate CC in the HSC niche [3]. Further studies into the relationship between p53, SPARC/Flower, and Ca²⁺ in the bone marrow niche could illuminate how cells compare their p53 levels in this microenvironment and enact their competitive mechanisms (Figure 2B).

A recent study demonstrated that low-grade inflammation provided a growth advantage to HSCs with overactivated Toll-like receptor–tumor necrosis factor receptor-associated factor 6 (TRAF6), a common cellular defect in MDS [69]. This mechanism hinged on the fact that TRAF6 HSCs signaled through noncanonical NF-κB, while WT HSCs utilized the canonical pathway. This study suggests that noncanonical NF-κB endows cells a growth advantage under inflammatory conditions, but whether this depends on the presence of WT cells is not known and therefore further studies are required.

**Competition in the Thymus: Competition for Interleukin-7 (IL-7) Maintains a Healthy Population of Thymocytes**

Lymphoid progenitors undergo differentiation into mature, naïve T cells in the thymus [70] and are constantly replenished from bone-marrow-derived precursors while also retaining self-renewal capacity. CC mediates old thymocyte replacement and the disruption of this mechanism leads to pathologies with genetic mutations and gene-expression profiles similar to those in T cell acute lymphoblastic leukemia (T-ALL) [64]. In these experiments, WT thymus grafted on recipients

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**Figure 2. Examples of Cell Competition (CC) in Cancer Stem Cell (CSC) Selection and Immune Cell Development.** (A) Hypothetical schematic diagram illustrating the evolution of CSCs encountering competitive environments. The cells with red nuclei are depicted as non-stem cancer cells and the cells without the red nuclei are depicted as CSCs. The light blue cells in this diagram represent the original CSCs, which divide to give rise to daughter CSCs (light blue) and non-stem cancer cells (light blue with red nuclei). Over time, the CSCs acquire mutations (and/or epigenetic changes). The CSCs with the 1st mutation (epigenetic change) are the dark-blue cells; the CSCs with the 2nd, 3rd, 4th, and 5th mutations (and/or epigenetic changes, or a combination of both changes) are the light-purple, dark-purple, black, and red cells, respectively. Consequently, over time, multiple mutant CSCs can exist in any given tumor. These CSCs compete with each other for resources. Under stressful conditions such as chemotherapy or irradiation, the competition among the CSCs becomes more intense and only a few CSCs can win because of mutations (and/or epigenetic changes) that give them a selective advantage under stressful situations. During the evolution of the tumor, the light-purple and the dark-purple CSCs survive chemotherapy; however, only the black CSCs survive radiation. These CSCs can acquire another mutations (epigenetic changes) (red CSC). In this way, the multiple competing CSCs evolve over time and accumulate additional mutations (and/or epigenetic changes) that provide a survival advantage over other CSCs. This is a continuous process of clonal evolution that is amplified by selective pressures (hypoxia, chemotherapy, irradiation, nutrient deprivation). (B) In the bone marrow, p53 mediates CC. Cells with lower p53 induce senescence of cells with higher p53. How hematopoietic stem cells (HSCs) communicate and compare their levels of p53 remains unknown, but studies highlighting the inhibitory role of Ca²⁺ provide a solution. WT, wild type. (C) In the thymus, thymocytes compete for interleukin-1 (IL-7), analogous to competition for Dpp in fly imaginal discs. Normally, long dwell times in the thymus reduce the efficiency with which older thymocytes can utilize IL-7 and are replaced by younger thymocytes. However, when younger thymocytes (blue) cannot utilize IL-7 due to a genetic deficiency, they are outcompeted by older thymocytes, and long dwell times in older thymocytes lead to genetic insult, which progresses to a T cell acute lymphoblastic leukemia (T-ALL)-like phenotype. (D) Cancer cells can utilize most of the metabolic substrates in the tumor microenvironment (TME). Thus, the TME is a nutrient-deprived, lactate-rich environment. This promotes the differentiation of immunosuppressive myeloid and lymphoid cells, which subsequently drives carcinogenesis. (E) Lymphocytes must come into physical contact with dendritic cells to become activated. The mechanism for this competition is not known, nor is the outcome of less-fit lymphocytes.
tested whether bone marrow progenitors from recipient mice with different genetic backgrounds could repopulate the thymus [64]. Signaling through IL-7, a hematopoietic growth factor, maintain the expression of Bcl2, an antiapoptotic protein. Environmental IL-7 drove CC and conferred a competitive advantage on progenitor cells with the ability to effectively utilize this factor [64]. Further supporting this model, young bone marrow progenitors from \textit{IL-7ra}−/− mice could not outcompete older, WT thymocytes (Figure 2C) [64]. The response to cytokines ensures proper immune cell development, homeostasis maintenance, and immunosurveillance for incipient cancer. However, the ability to enact specific pro/anti-inflammatory pathways in response to cytokine signals also depends on the ability of immune cells to meet their metabolic needs [71].

**Competition in the TME: Metabolic Suppression of Innate and Adaptive Immune Cells**

Competition for nutrients drives carcinogenesis and the TME provides a set of ‘rules’ for immune CC in the form of metabolic constraint [71]. Tumors that have metabolic winner phenotypes, like those with Myc overexpression, regulate local immune cell interactions. Myc gain-of-function mutations allow cancer cells to use a disproportionate amount of nutrients, which creates a nutrient-depleted, hypoxic, and acidic environment due to the depletion of glucose and oxygen and production of lactate [71,72]. Lactate has recently emerged as a pleiotropic immunoregulatory molecule with many functions, including the promotion of M2 macrophage gene expression through lactylation of histones [73]. M2 macrophages produce anti-inflammatory cytokines that promote the differentiation of tumor-infiltrating lymphocytes towards a T\textsubscript{reg} phenotype, which promotes immunosuppression in the TME (Figure 2D) [73]. Future studies attempting to answer the question of nutrient utilization and how subsequent changes in cell signaling affect fitness marker expression (i.e., Flower isoform expression) in the TME will lead to the identification of druggable targets and pathways to revert immunosuppression in the TME through novel CC mechanisms [74].

**Competition in the Lymph Node: Mechanical CC Maintains an Efficacious Cell Population**

Mammals employ immune mechanisms to monitor cellular fitness and prevent disease [75]. In the lymph node, T cells compete for space on dendritic cells for activation (Figure 2E) [76]. The mechanisms regulating this process remain enigmatic, but the importance of mechanosensitive pathways in T cell development and function is becoming increasingly recognized [77]. The Flower pathway can mediate CC via surface contact between cells in Drosophila and human [3]. Although Flower isoform expression has not been studied in human immune cells, selection through this pathway presents an interesting mechanism by which apoptosis of less-fit naïve T cells can be induced during education by lymph node dendritic cells. Education of the most competent naïve T cells promotes the development of a robust immune response and maintenance of immunosurveillance capabilities to fend off infection and incipient cancers, respectively. The discovery of Flower isoforms with distinct winner and loser potential in humans poses numerous questions about the complexity of immune CC. While cytokines allow different subsets of immune cells to compete, fitness fingerprints such as Flower isoforms could explain clonal CC.

**Concluding Remarks**

Studies have pointed towards the role of CC genes in cancer [4] and, as HIF-1α signaling under hypoxic conditions promotes aggressiveness [6], understanding of the transcriptional regulation of genes under hypoxia is highly relevant to understanding carcinogenesis in the context of CC. Novel omics technologies will undoubtedly contribute to the growing list of genes involved in CC (Table 1) and clarify their expression and role in other physiological and pathological conditions. Studies have revealed how many of these genes could function as novel therapeutic targets, regarding their tumor-promoting mechanisms or response to hypoxia [47,50–52]. It is also worth

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**Outstanding Questions**

As new proteins involved in CC are discovered, in what other ways do cells engage in competition under homeostatic, hypoxic, or malignant conditions?

As CC plays a role in ageing, can defective CC explain other risk factors for cancer at a molecular level?

What TME factors mediate CC in different tissue malignancies and how does it differ between different cancers and subtypes of cancers?

Does environmental oxygen play a role in CC beyond inducing cell signaling through HIFs? For example, does CC mediate the susceptibility of cells to reactive oxygen species?

What are the molecular details that mediate CC pathways in immune cell development, homeostasis, and function?

How do checkpoint blockade and immunotherapy affect CC in the TME?

How can we modulate specific CC pathways to enhance targeted anti-cancer therapies and give healthy cells an advantage over malignancy?
noting that the selection of CSCs contributes to tumor heterogeneity and subsequently impairs treatment efficacy, and selective modulation of CC might provide a way to overcome this challenge [8]. Last, a more complete understanding of the mechanisms regulating CC in immune cell populations and how these are influenced by pathological conditions could pave the way for novel immunotherapeutic strategies (see Outstanding Questions).

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