REVIEW

Lessons from development: A role for asymmetric stem cell division in cancer

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Abstract Asymmetric stem cell division has emerged as a major regulatory mechanism for physiologic control of stem cell numbers. Reinvigoration of the cancer stem cell theory suggests that tumorigenesis may be regulated by maintaining the balance between asymmetric and symmetric cell division. Therefore, mutations affecting this balance could result in aberrant expansion of stem cells. Although a number of molecules have been implicated in regulation of asymmetric stem cell division, here, we highlight known tumor suppressors with established roles in this process. While a subset of these tumor suppressors were originally defined in developmental contexts, recent investigations reveal they are also lost or mutated in human cancers. Mutations in tumor suppressors involved in asymmetric stem cell division provide mechanisms by which cancer stem cells can hyperproliferate and offer an intriguing new focus for understanding cancer biology. Our discussion of this emerging research area derives insight from a frontier area of basic science and links these discoveries to human tumorigenesis. This highlights an important new focus for understanding the mechanism underlying expansion of cancer stem cells in driving tumorigenesis.
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Introduction

The ability of stem cells to undergo asymmetric cell division as a way to self-renew is a tightly regulated process that occurs during development, tissue maintenance, and re-generation, and may be disrupted in hyperproliferative disease states such as cancer. Asymmetric cell division is typically restricted to stem cell populations where a need exists to preserve both a progenitor and a simultaneously generated differentiated progeny. This process is nicely exemplified in self-renewing tissues such as the epithelial layer of the human skin and intestine. Both organs possess stem cell pools that derive differentiated epithelia needed to maintain the function of the organ. The stem cell employs asymmetric division to maintain an appropriate census of daughter cells (or transient amplifying cells) and terminally differentiated cells. In the intestine, transient amplifying cells are characterized by their ability to amplify the epithelial population and likely undergo symmetric cell division to generate terminally differentiated cell popula-tions. In these self-renewing tissues, a critical balance between asymmetric and symmetric cell division is required to maintain tissue homeostasis. Asymmetric stem cell division is vital for this maintenance; thus, it is likely that tumors will develop if it is not properly regulated (Fig. 1a) (Morrison and Kimble, 2006). This dysregulation is consistent with the notion that expansion of a subpopulation of cancer cells harboring stem cell-like properties (cancer stem cells) may be the basis for propagating tumorigenesis. Though still controversial, there is growing acceptance that tumors may be dictated by this stem cell hierarchy; knowledge of how mutations in molecules that influence asymmetric stem cell division will provide insight into tumorigenesis. Mechanisms of asymmetric stem cell division have primarily been elucidated in invertebrate systems and encompass a number of molecules highly conserved in vertebrates (Morrison and Kimble, 2006; Knoblich, 2008; Doe, 2008; Yu et al., 2006).

A subset of proteins important for regulating asymmet-ric stem cell division are known tumor suppressors expressed in both invertebrate and vertebrate systems. Many of these tumor suppressors in *Drosophila melanoga-ster* have a role in tumor formation, and many of these genes have human homologues. Whether the function of these tumor suppressors in asymmetric stem cell division significantly contributes to cancer progression in verte-brate systems is not yet fully established; however, based on evolutionary conservation, it is intriguing to speculate that they may play an important role in human cancers.

Our intention is to review the role of four specific tumor suppressors involved in asymmetric stem cell division and discuss potential roles for their function in human cancer. Importantly, the lessons learned from the consequences of misregulating asymmetric stem cell division in developmen-tal systems can inform the emerging research focus on the mechanism underlying cancer stem cell expansion as it relates to tumor progression.

Much of what is known about the molecular mechanism underlying asymmetric cell division is based on examination of developmental systems in *Drosophila*. Through elegant studies of the *Drosophila* neuroblast and germline cells, we know that the polarity and spindle orientation of the stem cell—the major determinants of asymmetric stem cell division—are governed by factors that are both intrinsic and extrinsic (Knoblich, 2008; Doe, 2008; Yu et al., 2006; Bilder, 2004). For example, in the fly neural stem cells or "neuroblasts," segregation of intracellular proteins to the apical or basolateral region of the cell determines whether a stem cell asymmetrically divides, giving rise to both a stem cell and a differentiated daughter cell, or if it symmetrically divides to produce two stem cells (Lee et al., 2006; Bowman et al., 2006). In contrast, *Drosophila* germline cells rely on extrinsic factors within the stem cell niche to define the orientation of the mitotic spindle, which is critical for proper cell division (Yamashita et al., 2003, 2007). In addition, proper cell division of the self-renewing, polarized mouse intestinal epithelium is regulated by the positioning of the mitotic spindle (Fleming et al., 2007). From these two model systems, it is clear that both the orientation of the spindle and segregation of polarity components ultimately deter-mine whether a stem cell will asymmetrically or symmetri-cally divide.

While it is yet unclear if these two well-defined examples of asymmetric stem cell division directly apply to division of human stem cells or to a putative cancer stem cell population, they offer a testable model to shape our investigation of the link between mammalian cancer and stem cell biology. Moreover, emerging evidence from the fly and mouse suggests that mutations in particular tumor suppressors that govern asymmetric stem cell division disrupt the normal ratio of stem cells to differentiated cells and contribute to unregulated proliferation of tumors. The following tumor suppressors—Adenomatous polyposis coli (Apc), Lethal giant larvae (Lgl), Brain tumor (Brat) and p63—display a novel and intriguing link between inverte-brates and vertebrates and the regulation of asymmetric stem cell division in both tissue stem cells and tumorigenesis.

Adenomatous polyposis coli: A novel role in stem cell mitotic spindle orientation

APC is a human tumor suppressor with a well-documented role in the Wnt signaling pathway, primarily governing proliferative division of stem cells (Morin et al., 1996; Rubinfeld et al., 1997). In this capacity mutations in APC result in upregulated cellular proliferation and tumor formation. Not surprisingly, this gene is mutated or sup-pressed in a number of cancers, including hepatoblastoma (Oda et al., 1996), medulloblastoma (Huang et al., 2000), adult T-cell leukemia (Yang et al., 2005), and most notably in colorectal cancer where nearly all forms harbor mutations in APC (Su et al., 2000). The dominant association between the
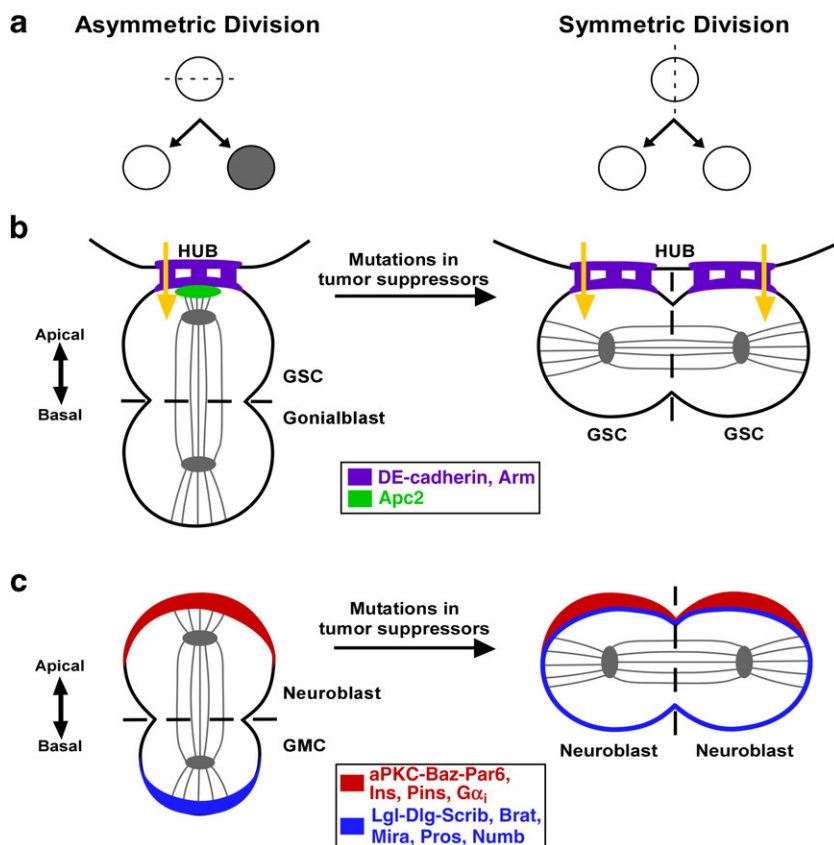
Figure 1   Model illustrating how mutations in factors regulating asymmetric stem cell division in *Drosophila* neuroblast and germline cells result in uncontrolled expansion of stem cells. (a) Stem cells can undergo asymmetric division giving rise to both a stem cell (white) and a differentiated cell (gray), or symmetric division that produces two stem cells. (b) In the *Drosophila* germline stem cells (GSC) asymmetric cell division requires Adenomatous polyposis coli-2 (Apc2; green oval) and its partners DE-cadherin and Armadillo (Arm)/β-catenin (purple) at the HUB cell interface to support proper mitotic spindle orientation, as well as additional microenvironmental extrinsic cues (yellow arrows). This yields one GSC and one Gonialblast. However, mutations in tumor suppressors result in symmetric cell division that yields two GSCs and consequent hyperproliferation of the stem cell pool. (c) Asymmetric division of the *Drosophila* neuroblast requires correct localization of the apical complex (red crescent), atypical Protein Kinase C-Bazooka-Par6, Inscuteable, Partner of Inscuteable, and $G\alpha_i$ (aPKC-Baz-Par6, Ins, Pins, and $G\alpha_i$), and basal restriction of Lethal giant larvae-Discs large-Scribble, Brain tumor, Miranda, Prospero, Numb (Lgl-Dlg-Scrib, Brat, Mira, Pros, Numb; blue crescent). Normal asymmetric neuroblast division results in self-renewal of the stem cell and production of a differentiated ganglion mother cell (GMC). Mutations in tumor suppressors cause mislocalization of the basal proteins and thus generate two neuroblasts resulting in uncontrolled stem cell proliferation.

APC and the Wnt signaling pathway overshadows the multiple functions of the APC protein (reviewed in Hanson and Miller, 2005). The human APC gene encodes a large multidomain protein that can interact with a number of partner proteins (Hanson and Miller, 2005). As such, APC has been demonstrated to interact with microtubules, suggesting a role in cell migration (Hanson and Miller, 2005; Dikovskaya et al., 2007; Kroboth et al., 2007). Additionally, it has been shown to be involved in regulating mitotic spindle assembly and chromosome segregation (Kaplan et al., 2001). Further, APC has also been described to participate in regulation of cell cycle progression and apoptosis (Dikovskaya et al., 2007; Baeg et al., 1995). Although each of these roles attributed to APC has potential importance in tumor progression, its role in asymmetric stem cell division most intriguingly suggests an important function in modulating expansion of cancer stem cells. Recently, Apc was discovered to be a component of the centrosome complex of *Drosophila* germline cells. Here it functions in establishing asymmetric stem cell division,

distinct from its role in Wnt signaling. In this capacity, mutations in APC could effectively expand early cancer cells or a putative cancer stem cell pool.

In the male *Drosophila* germline, Apc2 anchors the mother centrosome of the germline stem cell so that it is adjacent to the hub cell. The hub cell provides extrinsic, supportive cues to the stem cell that orient the mitotic spindle. The contact surface between the hub cell and the germline stem cell is also marked by concentrated levels of proteins known to interact with Apc2 (Fig. 1b). This interaction localizes Apc2 to the interface between the hub germline stem cell, fixing the mother centrosome at one pole of the stem cell and allowing the daughter centrosome to migrate to the opposite side of the germline stem cell (Fig. 1b). This movement allows the correct orientation of the mitotic spindle (Yamashita et al., 2007; Penman et al., 2005). In *Drosophila*, deletion of both *Apc* genes results in misorientation of centrosomes and the mitotic spindle, and consequently disrupts asymmetric stem cell division. At the tissue level,

the phenotypic consequence is hyperproliferation of germ-line stem cells at the expense of differentiated cells (Yamashita et al., 2003).

To date, the most well investigated role of *APC* has been its function as a tumor suppressor. However, these new findings in *Drosophila* reveal a novel role for *Apc* in asymmetric stem cell division, which may intensify its role in tumor progression. It is easy to speculate that APC in humans may also be essential for spindle orientation and proper asymmetric division of tissue stem cells. In this scenario, mutations in *APC* would also lead to increased numbers of cancer stem cells. Investigation of APC's role in the regulation of asymmetric stem cell division represents an important future focus of human cancer biology.

Lethal giant larvae: Regulating stem cell polarity and differentiation

Normal asymmetric neuroblast division results in self-renewal of the stem cell and production of a differentiated ganglion mother cell (GMC; Fig. 1c). The correct positioning of both apical and basal protein complexes is critical for proper asymmetric cell division. Mutations in tumor suppressors cause mislocalization of these basal proteins, generating two neuroblasts that result in uncontrolled stem cell proliferation (Fig. 1c). Three *Drosophila* tumor suppressor genes, *lethal giant larvae* (*lgl*), *discs-large* (*dlg*), and *scribble* (*scrib*), act in a common pathway in the mitotic neuroblast to establish the asymmetrically localized cortical basal complex and ultimately influence asymmetric stem cell division. Specifically, Lgl interacts with the apical complex shown in Fig. 1c—atypical protein kinase C-Bazooka-Par6, Inscuteable, Partner of Inscuteable, and Gα$_i$ (aPKC, Ins, Pins, and Gα$_i$). In addition, it restricts the localization of active basal complex molecules (Wirtz-Peitz et al., 2008; Betschinger et al., 2003) and this is illustrated in mutants for Lgl that possess abnormal targeting of the basal complex proteins Miranda, Prospero, and Numb (Mira, Pros, Numb) (Fig. 1c; Lee et al., 2006; Betschinger et al., 2003).

Proper location and function of Lgl is critical to the prevention of tumor formation. Lgl mutants do not asymmetrically divide but instead produce two stem cells at the expense of neuronal populations. This ultimately leads to expansion of the stem cell population and subsequent formation of brain tumors (Lee et al., 2006). Supporting this role in tumor suppression and the regulation of proper asymmetric cell division, loss of mouse Lgl1 results in disrupted asymmetric cell division and a brain tumor phenotype (Klezovitch et al., 2004). Tumor initiation and cancer progression in humans may, in part, be driven by misregulation of Lgl homologues. Strikingly, a human homologue of *lgl*, *HUGL-1*, is lost in many solid tumors and is strongly correlated with advanced stages of malignant melanoma, colorectal cancer, and endometrial cancer (Kuphal et al., 2006; Schimanski et al., 2005; Tsuruga et al., 2007). In addition, recent work examining *HUGL-1* in human hepatocellular carcinoma reveals that the mRNA is frequently mutated by aberrant splicing. This renders the protein inactive and functions as an important mediator of hepatocellular carcinoma progression (Lu et al., 2009).

HUGL-1 is highly structurally and functionally conserved, as observed by the ability of *HUGL-1* to rescue *Drosophila lgl* mutants (Grifoni et al., 2004). Both HUGL-1 and another Lgl human homologue, HUGL-2, directly interact with the human aPKC-Par6 protein complex (Yasumi et al., 2005) (Fig. 1c), behaving just as Lgl does in *Drosophila*. Further, inhibition of this binding induces disorganization of the mitotic spindle during normal mitosis and results in aberrant cell division (Yasumi et al., 2005). Even further evidence from the examination of the behavior of *Drosophila* polarity proteins in human ovarian cancer epithelium reveals that Lgl function is conserved (Grifoni et al., 2007) between flies and humans. This suggests that both HUGL-1 and HUGL-2 play active roles in establishing polarity and spindle orientation for asymmetric cell division of human epithelial stem cells and, when mutated, may result in tumor initiation and/or expansion of cancer stem cells. This intriguing possibility has yet to be directly investigated.

Brat: A critical protein for balancing stem cell self-renewal and proliferation

The *Drosophila* larval neuroblast tumor suppressor with emerging relevance to human cancer is the newest member of the basal complex, "Brain tumor" (*brat*, Fig. 1c). Brat, like Prospero, Miranda, and Numb, is asymmetrically localized during neuroblast cell division to the basal cortex (Fig. 1c). Brat removal results in extensive proliferation of larval neuroblasts at the expense of differentiated neurons and generates tumors (Lee et al., 2006; Betschinger et al., 2006). Further investigation revealed that Brat mutants exhibit and uncontrolled expansion of transient amplifying cells, resulting from a failure to progress through the cell cycle (Bowman et al., 2008).

The structure of Brat contains clues about its role in asymmetric stem cell division and control of tissue proliferation. Brat contains an NHL domain, an evolutionarily conserved motif found in proteins that posttranscriptionally regulate gene expression via mRNA binding and inhibition of translation. Indeed, in *Drosophila*, Brat is thought to be a posttranscriptional inhibitor of Myc (Sonoda and Wharton, 2001). Myc transcriptionally regulates a variety of cell cycle and cell growth genes to support proliferation as opposed to differentiation. Interestingly, dMyc translation is de-repressed in cells without Brat. Studies in the mouse neocortex support this finding where TRIM32, a Brat homologue, is asymmetrically localized in one of the two daughter cells and becomes upregulated during neuronal differentiation (Schwamborn et al., 2009). TRIM32 has dual roles both as a tumor suppressor functioning to degrade cMyc and in asymmetric cell division where it is asymmetrically located and activates certain microRNAs important for stem cell self-renewal (Schwamborn et al., 2009). Although a role for TRIM32 in asymmetric stem cell division in human cancers has not been described, its overexpression in human head and neck squamous cell carcinoma samples highlights its activity as an E3 ubiquitin ligase (Horn et al., 2004; Locke et al., 2009; Albor and Kulesz-Martin, 2007; Boulay et al., 2009), and it has been suggested to potentially be a cause of cancer. This analysis is complicated by functional redundancy in the mouse and human systems, as both species also

express TRIM2 and TRIM3, which are also human Brat homologues. To this end, loss of heterozygosity of the tumor suppressor TRIM3 has recently been implicated in human malignant gliomas (Boulay et al., 2009), where it is intriguing to speculate that it may have a role in asymmetric cell division and formation of a potential cancer stem cell population. Clearly, additional studies are required to establish a role for the Brat family homologues in asymmetric stem cell division in humans. However, the intriguing evidence from mouse and fly suggests that Brat is certainly multifunctional. Importantly, like the APC tumor suppressor, Brat is most well-known for an early described function in tumorigenesis that overshadows a potentially important role in asymmetric stem cell division specifically contributing to cancer stem cell expansion.

p63: A novel role in stem cell spindle orientation and proliferation

While numerous studies have examined asymmetric cell division in invertebrates, recent reports have uncovered a role for this process in mammalian systems. For example, *p63*, a closely related member of the *p53* tumor suppressor family, is highly expressed in asymmetrically dividing stratified epithelial cells that are susceptible to cancer (Yang et al., 1999). *p63* has been implicated and is now classically described as a master switch regulator of epithelial stem cell commitment, maintenance, and differentiation (Koster et al., 2004).

In both mouse and human, full-length *p63* is spliced into multiple isoforms and all of the resulting protein products have distinct, complex roles described in both oncogenesis and tumor suppression, depending on the tissue- and/or tumor-specific context for which they are examined (Westfall, 2004; Deyoung, 2007). For example, ΔNp63α is upregulated in breast, gastric, cholangiocarcinoma, and chronic myeloid leukemia, but not in leukemia, where the *TAp63* isoform is overexpressed. In addition, molecular interactions between the isoforms, which limit the activity of one another, have also been described, further complicating analyses of these proteins (Deyoung, 2007).

Interestingly, the *p63* transcription factor was recently implicated in mitotic spindle orientation during asymmetric epidermal stem cell division, one of the first studies to examine *in vivo* asymmetric stem cell division in a mammalian system (Lechler and Fuchs, 2005). Accordingly, its role in establishing spindle orientation may be related to the proliferative potential of dividing cells, a critical mediator in cancer progression. Stratified epithelial cells undergo symmetric and asymmetric divisions during normal development. Under these conditions, the mitotic spindle orients perpendicular to the basement membrane and mammalian homologues of the apical complex localize to the apical cortex prior to cell division. In contrast, in a *p63* null mouse mutant, the spindle is parallel in orientation and the complex is mislocalized (Lechler and Fuchs, 2005), resulting in the disruption of tissue organization.

In general, misregulation of any of the *p63* isoforms is implicated in human tumor formation; however, a definitive and consistent correlation between expression of the *p63* isoforms and either cancer initiation or progression remains controversial (Westfall, 2004; Deyoung, 2007). Many human cancers harbor an overexpression of one or more isoforms of *p63*, supporting an oncogenic role in tumorigenesis. In addition, some studies indicate that overexpression of *p63* can cause an epithelial-to-mesenchymal transition, leading to an upregulation of genes involved in cell migration and invasion during metastasis (Koster et al., 2006). Interestingly and conversely, human bladder and urothelial cancers exhibit loss of *p63*, supporting a tumor suppressive role (Koga et al., 2003; Park et al., 2000). In either situation and in light of the mouse and fly data, it is possible that either over- or underexpression of *p63* may contribute to aberrant spindle orientation in the stem cell population. Clearly, the existence of multiple *p63* isoforms complicates our appreciation of its role in asymmetric stem cell division, although the extensive descriptions of the function of p63 in epithelial stem cell biology combined with the recent spindle orientation studies in the mouse (Lechler and Fuchs, 2005) provide an undeniable basis for further examination of the role of p63 in spindle orientation in asymmetric stem cell division.

Conclusion

In any self-renewing tissue, maintaining a balance between stem cells and their differentiated progeny depends on

Table 1 A summary of the investigations of mutations in APC, Lgl, Brat, and p63* from fly, mouse, and human cancers and their potential link to asymmetric stem cell division (ACD)

Roles for tumor suppressors in asymmetric stem cell division and cancer			
	Fly	Mouse	Human
Apc	ACD linked to tumor formation	ACD; Tumor formation	Tumor formation
Lgl	ACD linked to tumor formation	ACD linked to tumor formation	ACD linked to tumor formation
Brat	ACD linked to tumor formation	Tumor formation	ACD; Tumor formation
p63	ND	ACD linked to tumor formation	Tumor formation

ACD linked to tumor formation, the protein has defined role in asymmetric stem cell division that has been linked experimentally to tumor formation. ACD; tumor formation, the protein has defined role in asymmetric stem cell division as well as tumor formation, but these functions have not been experimentally linked. Tumor formation, the protein has defined role in tumor formation, but a link to ACD has not yet been described.
  * The full-length p63 isoform is referred to here. *ND*, not determined, as a full-length, p63 homologue has not been identified in *Drosophila*. This table encompasses the investigations cited in this review.

tightly regulated asymmetric stem cell division (Morrison and Kimble, 2006). Recent investigations in invertebrate and vertebrate systems have established that successful asymmetric cell division is dependent on asymmetrically localized proteins and mitotic spindle orientation. Proper execution of these two cellular programs functions in cell fate determination to control whether a cell assumes either a stem or a differentiated identity. When mutations occur in genes involved either directly or downstream of the intrinsic or extrinsic cues governing these mechanisms, the resulting asymmetric cell division is abnormal and leads to uncontrolled amplification of stem cell populations.

The tumor suppressor proteins described in this review represent just four examples of molecules that regulate developmental or adult homeostatic asymmetric cell division, but we highlight the critical need for further investigations regarding the relevance of these tumor suppressors in disrupting asymmetric stem cell division in human cancer. Indeed, a precedent for such translational research is ongoing in work addressing *Drosophila* cell polarity determinants and proliferation control, and their implications on mammalian cancer progression (Bilder, 2004; Grifoni et al., 2004; Grifoni et al., 2007; Caussinus and Gonzalez, 2005; Gonzalez, 2007; Hawkins and Russell, 2008). Studies emerging from this nascent field may implicate a critical and novel role for these proteins in early tumor initiation and asymmetric stem cell division of a putative cancer stem cell population.

Direct links between tumorigenesis and asymmetric stem cell division do exist in mutants of the well-documented mammalian tumor suppressor, APC, the p53 family member, p63, and the *Drosophila* polarity protein, Lgl (summarized in Table 1). Mutations in these tumor suppressors are associated with advanced tumor progression, metastasis, and poor patient prognosis, yet their function in aberrant asymmetric stem cell division in cancer has not been fully explored despite the amount of overwhelming evidence derived from lessons in developmental biology. The findings we present here likely represent but a few examples of how clues from developmental biology can illuminate key insights into cancer cell biology, specifically, the study of aberrant asymmetric stem cell division of human tissue stem cells and the expansion of a cancer stem cell pool in tumorigenesis. Clearly defining a role for the underlying mechanism driving aberrant expansion of a cancer stem cell pool provides further justification for targeting the cancer stem cell as an important therapeutic approach for treatment of disease.

Acknowledgments

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