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Evolution of Cancer Stem Cells

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1. Introduction

Theodosius Dobzhansky (1973) proclaimed, "Nothing in biology makes sense except in the light of evolution." But how can evolution make sense of something as manifestly maladaptive as metastatic tumors, leukemia, and lymphomas? How does evolution explain cancers invading and destroying vital tissues? How are "[u]ncontrolled cell proliferation" (Sherr, 1996) and "[g]enetic lesions that disable key regulators" (Sherr, 2000) reconciled with evolution?

Possibly cancers appeared unbidden in vertebrates without having evolved! This possibility cannot be dismissed out of hand, since animals on invertebrate branches of the metazoan tree, Lophotrochozoa, Ecdysozoa, bilaterians, coelomates, and deuterostomes develop non-malignant growths spontaneously *but not cancers* (Sutherland, 1969; Matz, 1969).

On the other hand, *induced* malignancies in *Drosophila* (Gateff & Schneiderman, 1969), "[a]lthough not naturally occurring" (Gonzalez, 2007), and aberrant patterns of cell death and changes in specification in *Caenorhabditis elegans* suggest that cryptic cancers exist in invertebrates. Moreover, widely distributed molecular homologues (i.e., genomic equivalents) in metazoans point to fundamentally "conserved" or "canonical, core pathways" common to human cancers and invertebrate tissues (Potts & Cameron, 2011). For example, "ancestral forms of myc and max [onco]genes … [appear in] the early diploblastic cnidarian *Hydra*" (Hartl et al., 2010), and a portion of an acute myelogenous leukemia gene (AML1) has 67% identity over 387 base pairs with 69% amino acid identity with the *Drosophila* segmentation gene *runt* (Erickson et al., 1992). In addition, cell death is induced by genotoxic stress in *Drosophila* as it is in cancers (Jin et al., 2000).

Other molecular evidence also supports the notion of cancer's evolution. For instance, evolutionary creativity, competition, and selection are suggested by redundancy of the human p53 cancer suppressor gene known as the "guardian of the genome" (Levine & Oren, 2009). Moreover, the planarian homologue of human p53 "functions in stem cell proliferation control and self-renewal" (Pearson & Alvarado, 2010); "ancestral forms" of p53 "mediate … multiple stress responses in the soma" of *C. elegans*; and "a primordial p53 ancestor gene which appeared early in phylogenesis" is found in the squid, *Loligo forbesi* (Schmale & Bamberger, 1997).

More direct evidence for cancers in invertebrates has emerged from efforts to evaluate effects of pollutants on animals. For example, a transmissible sarcoma that breaks out epizootically in Maryland soft-shell clams, *Mya arenaria*, would seem to be infectious but may also be synergistically promoted by contamination with the pesticide chlordane (Farley

et al., 1991). Herbicide contamination is also correlated with outbreaks of gonadal neoplasms (seminomas and dysgerminomas) and catastrophic declines of reproduction in softshells (Gardner et al., 1991a) and in hard shell clams (*Mercenaria spp.*) (van Beneden, 1994). Similarly, the eastern oyster, *Crassostrea virginica*, develops neoplasm at multiple sites when exposed to suspensions of Black Rock Harbor sediments known to contain "genotoxic carcinogens, co-carcinogens, and tumor promoters." And winter flounders fed on the blue mussel, *Mytilius edulis,* raised on contaminated sediments develop renal and pancreatic neoplasm "demonstrating trophic transfer … up the food chain" (Gardner et al., 1991b). The carcinogens in polluted effluvia, such as polynuclear aromatic hydrocarbons, chlorinated hydrocarbons, pesticides, and/or metals sequestered by aquatic bivalves induce liver neoplasm in teleosts and in human beings (Stegeman & Lech, 1991).

Animals, larvae, and embryos also play tricks on cancers that seem rooted in an evolutionary past. For example, an aqueous extract from the common clam (*Mercernaria mercenaria*) promotes regression in viral induced tumors in hamsters and melanomas in mice (Li et al., 1972). The soft coral, *Sarcophyton glaucum,* produces an anti-tumor agent effective against the development of chemically induced mouse skin and rat colon carcinoma (Narisawa et al., 1989). And receptors for the snail hemagglutinin HP present "on leukaemic lymphocytes … in combination with conventional surface marker analysis provides a new important tool for monitoring patients with CLL [chronic lymphocytic leukemia]" (Hellström et al., 1976).

Another argument in favor of cancers' evolution relies on reminiscences of recapitulation, namely that presumptive ancestral types of animals and younger human beings foster fewer malignancies than adult human beings. For instance, in *Drosophila,* "metastases nearly always occur in transplanted [adult] hosts rather than in the larva in which the primary tumours first arose" (Gonzalez, 2007). In human beings, the "overall incidence of cancer in persons under 15 years of age is one-thirtieth that of the population as a whole … Indeed, most pediatric cancers consist of leukemias, lymphomas, and sarcomas … In contrast, more than 80% of adult cancers in the United States are carcinomas … and 8% are hematopoietic with a higher preponderance of myeloid leukemia than is observed in children … Carcinomas are rare in persons under age 30, rising exponentially in incidence thereafter" (Sherr, 1996).

Rather than never having had cancers, invertebrates and human young seem to have evolved successful strategies of cancer suppression, at least before anthropogenic pollution lowered the bar to tumorigenicity. Conceivably, in human beings, inclusive fitness (the sum of advantages that project a living thing's progeny into the next generation) pushed cancers generally and carcinomas and myelomas in particular into adult years beyond the reproductive prime.

On balance, evidence of cancers having evolved is abundant and robust. If Dobzhansky is right, therefore, the light of evolution will yet illuminate the biology of cancers (Shostak, 1981, 2007-8; Zimmer, 2007).

2. Studying cancers' evolution

In order to avoid ambiguity, the sense in which the "evolution of cancer" is used here must be distinguished from the sense in which the "evolution of cancer" is typically used in the oncology literature. Oncologists typically equate "cancer's evolution" with "tissue independent [gene-expression] signature[s] associated with metastasis" (Ramaswamy et al.,

2003; van 't Veer et al., 2002) or mutational patterns (aka spectra) appearing throughout a cancer's development from a single cell. The "cancer genome … [is said to leave] an archaeological record bearing the imprint of [mutagenic and DNA repair] processes" (Stratton, 2011).

This developmental/genetics' sense of "cancer's evolution" is *not* the sense in which the term is used here. Here, "cancers' evolution" refers to cancers' proterozoic origins and subsequent history of adaptations leading to contemporary malignancies.

Phylogenomic analysis would be the method of choice for studying cancers' evolution in the sense intended here, and, no doubt, such an analysis will be feasible when "[o]ver the next 5 to 7 years … tens of thousands of cancer genomes will be sequenced … an essentially complete set of cancer genes … revealed … [and] the complete catalog of somatic mutations provided by the sequence of the cancer genome" (Stratton, 2011). Today, however, cancer's phylogenomics are inaccessible. Rather, spotty spectra of mutations all but obliterate the trail of cancers' genomic phylogeny. Instead of genomic coherence, clonal diversity in cancers is found in copy number DNA profiling (Notta et al., 2011) and multiplexing fluorescence in situ hybridization (Anderson et al., 2011). The results of single-nucleus sequencing in two ductal human breast cancers and paired liver carcinomas show that "metastatic cells arise late in tumour development" and that tumors grow by "punctuated clonal evolution" with few persistent intermediates (Navin et al., 2011). Making matters worse, rather than translocations at oncogenic sites producing cancers (Bohr et al., 1987; Croce, 2008), single catastrophic events lead to massive chromosomal rearrangements, and chaotic chromosomal architecture (Stephens et al., 2011; Berger et al., 2011; Tubio & Estivill, 2011). Furthermore, outside the cellular mainstream, "cancer can be initiated in cells … [with] long-term reconstituting ability … [and] self-renewal capacity" (Visvader, 2011); metastases may be formed where malignant niches recruit cells from local or circulating sources (König et al., 2005); and tumors may arise from long dormant cancer initiating cells (CICs) "with a metastatic potential … [to] disseminate … even at a premalignant stage" (Ansieau et al., 2008). Ultimately, "it is unclear how best to assess the effects of new genetic lesions on … growth, differentiation, tumorigenicity and functionality" (Pera, 2011).

Cancers' evolution is thus pursued here the old-fashioned way, by following Charles Darwin's lead and asking, "[without supposing] that the modifications were all simultaneous … [how would d]ifferent kinds of modification … serve the same general purpose" (Darwin, 1958 [1872])? In the case of cancer, the notion of a "general purpose" is epitomized by cancers' stem cells invading normal tissues and destroying their cells while metastasizing, and, growing elsewhere in the organism to the same effect. Darwin's question becomes, therefore, what "kinds of modification" would produce cancers' stem cells?

Two distinctly different possible answers stand out: (1) Cancers' stem cells arose from normal self-renewing cells which added invasiveness, destructiveness, and metastasis to their repertoire of cell behaviors; (2) Cancers' stem cells and normal tissues' stem cells arose through competition within cell populations in response to evolutionary pressures and adaptive advantages.

3. Is the stem cell the root of cancers' evolution?

Did a rudimentary stem cell provide the ancestral branch or common root of cancers' stem cells? The cancer stem cell theory encapsulates this idea by proposing that normal tissues

and cancers converge on stem cells. The problem is to find common ground among the many cells identified as both normal and cancer stem cells.

In general, stem cells fall into three or four categories: adult stem cells, separated into organ stem cells (OSC aka somatic stem cells) and hematopoietic stem cells (HSC), germ stem cells (GSC), and embryonic stem cells (ESC). Each of these has its malignant complement: cancer stem cells (CSCs) in solid tumors complement OSCs in solid organs; malignant HSCs (malHSCs) in leukemia, lymphoma, and related cancers represent the malignant counterpart of HSCs of normal blood and lymph; malignant GSCs (malGSCs) in testicular and ovarian cancer are the malignant counterparts of oogonia and spermatogonia; malignant embryonic stem cells (malESCs), thought to be present in small cell cancers and other malignancies, resemble (hypothetical) retained or reproduced post-embryonic ESCs.

The list is easily expanded by adding other cells called stem cells (see below), but the list is also contracted by squeezing one or another so-called stem cell into the above categories. For example, mesenchymal stem cells (MSCs) resemble HSCs or marrow stem cells (also MSCs) in several ways including differentiating as skeletal muscle, fat, cartilage, or bone (Young et al., 2004). Even GSCs are easily absorbed in the HSC category, inasmuch as both types of stem cells are derived embryonically from wandering, infiltrating, and colonizing cells (see Shostak, 1991), and both are especially plastic in the range of cells ultimately differentiating from their stock.

The most problematic stem cells are the ESCs. Whether they exist in adults at all is uncertain, although OSCs and HSCs are sometimes said to be virtual ESCs. This claim would seem vastly exaggerated, since neither OSCs nor HSCs possess ESCs' prime virtue of differentiating into cells of all three germ layers. Rather, ESCs are subsumed by germ layers in early development and disappear entirely in the parenchyma and stroma of adult organs during morphogenesis. OSCs and HSCs then emerge fresh in adult tissues.

Some similarities between the behavior of embryonic and cancer cells suggested that cancers originated from leftover or restored embryonic cells, but, historically, the alternative idea that stem cells produced metastases took precedence. This idea is traced to Rudolf Virchow. Even if he didn't use the term, he clearly attributed metastasis to unique proliferative cells as distinguished from differentiated cells. He wrote, "the transference … disposes different parts to a reproduction of a mass of the same nature as the one which originally existed," although, later, he added that he "must confess" that he can do no more than "allow it to be possible that the diffusion by means of vessels may depend upon a dissemination of cells from the tumours themselves" (Virchow, 1971 [1863]).

Evidence of stem cells as a source of cancer was indecisive until it became overwhelming: injected single murine embryonal carcinoma cells (ECCs) produced teratocarcinomas (Kleinsmith & Pierce, 1964); cells of a non-T cell line produced human acute lymphoblastic leukemia (ALL) (Kamel-Reid et al., 1989); "primitive hematopoietic cells" as opposed to "committed progenitors" produced human acute myelogenous leukaemia (AML) (Lapidot et al., 1994; Bonnet & Dick, 1997); small "CD44$^+$CD24$^{-/low}$ Lineage$^-$ [cell] populations" uniquely formed breast cancers (Al-Hajj et al., 2003), and as few as one hundred CD133 positive cells from human brain cancers recreated "classical histopathological features of [the patient's original] tumour type" in immuno-compromised mice (Singh et al., 2004). The idea of "a small subpopulation of leukemic stem cells that possess extensive proliferative capacity and the potential for self-renewal" was quickly generalized to a cancer stem cell theory according to which cancers were stem cell-supported and metastases were stem cell-dependent (see Lobo et al., 2007).

Cancer stem cell theory's great attraction was the explanation it offered for two of malignancy's great enigmas, namely, recurrence and the enhanced resistance to chemo- and radiation therapy displayed by the returning cancer (O'Brien et al., 2007–8; Gilbert & Ross, 2009; Ropolo et al., 2009). The explanation was seductively simple: Chemo- and radiotherapies targeted the abundant, rapidly dividing non-stem cancer cells, while rare stem cells dividing at low rates escaped the effects of treatment and regenerated the cancer. Moreover, since selection for a predisposition to resistance was also in play, the recurrent cancers had enhanced resistance to similar therapies. The prognostic and therapeutic implications were unmistakable: the fewer stem cells, the more promising the prognosis; eradicating a cancer depended on eliminating all stem cells.

Cancer stem cell theory soon launched a virtual cancer stem cell industry. Its business was to define, find, and isolate cancer stem cells for the purpose of destroying them.

3.1 Defining stem cells

Stem cells' principal attribute is self-renewal, the ability to maintain or expand a specific population of stem cells through cell division while also producing cells that give rise to a tissue's or a cancer's bulk (characteristic) cells. Self-renewal takes place in either a maintenance or expanding mode. In normal adult tissues at homeostasis, OSCs, HSCs, GSCs, and possibly ESCs undergo maintenance self-renewal by dividing asymmetrically thereby giving rise to different sibling cells (Chartier et al., 2010). Generally, one cell replaces the stem cell and one enters a "transit amplifying" (TA) pathway of division, terminal differentiation, and disposal. CSCs, malHSCs, malGSCs, and malESCs also perform asymmetric division, producing stem and bulk tumor cells, (Norton, 2007-8; Powell et al., 2010; Quyn et al., 2010), but following a premalignant transition and in growing cancers some stem cells also undergo expanding self-renewal by symmetric division (Tomasetti & Levy, 2010) thereby giving rise to identical self-renewing sibling cells, enlarging the cancer stem cell population and contributing to tumorigenesis.

The difference in the mode of self-renewal places cancer and normal stem cells on a sliding scale rather than separate stem cell branches. Some other differences between normal and cancer cells are also differences of degree rather than kind. For example, ECCs produce benign cells and normal tissues within teratocarcinomas (Pierce, 1974) and differentiate into normal mammary epithelium in epithelial-free mammary fat pads of athymic (aka nude) mice when mixed with mouse mammary epithelial cells (Bussard et al., 2010a).

But defining stem cells by self-renewal may still not homogenize them. According to the "gold standard assay" (Clarke et al., 2006), putative stem cells renew themselves while giving rise to tumors following transplantation in vivo and to tumor-like nodules following serial passage in vitro. The assay breaks down, however, for identifying stem cells that resist transplantation and nodule formation.

A different assay identifies stem cells without recourse to transplantation or passage. This assay relies on the retention of label by cells in long-term pulse-chase experiments and the premise that stem cells divide rarely. For example, putative smooth muscle stem cells of the uterine myometrium are labeled in perpetuity by a pulse with the DNA nucleotide-mimic 5-bromo-2–deoxyuridine (Szotek et al., 2007). These "label-retaining cells" (LRCs) are also found in the endometrial epithelium and stroma (Chan & Gargett, 2006), intestinal absorptive and gland epithelium, mucous epithelium of the tongue (Fellous et al., 2009), mammary epithelium (Booth et al., 2008), neurons (Das et al., 2003), satellite reserve skeletal

muscle cells (Shinin et al., 2006; Conboy, Karasov, & Rando, 2007; Kuang et al. 2009), and in cancers of the breast (Trosko, 2006; Bussard et al., 2010b) and intestine (Barker et al., 2008). LRCs are also found in yeast (Klar, 1987), bacteria, plants, fungi, the round worm, *Caenorhabditis elegans,* the fruit fly, *Drosophila,* and elsewhere (Tajbakhsh et al., 2009).

A sluggish division rate might represent an anti-mutation adaptation since delaying cell division provides an opportunity for correcting replication errors and performing DNA repair. Thus, in cancers' stem cells, the post- and pre-mitotic gaps (see below) function as checkpoints for DNA damage and damage response signaling networks (Bao et al., 2006; Kuntz & O'Connell, 2009). Lengthening these gaps and suspending progress through the cycle, therefore, would aid in repairing damaged DNA (Wang et al., 2009). On the other hand, cells with damage too severe to be adequately repaired are dispensed without replicating their errors.

But if the LRC divided repeatedly after acquiring the labeled DNA precursor, the cell might have remained labeled because it retained labeled "immortal strands" of DNA while casting off unlabeled DNA strands replicated during the chase phase of the experiment (Cairns, 2006). The retention of "immortal strands" of DNA would also seem an anti-mutation adaptation, since it would help keep stem-cell DNA pristine by reducing opportunities for errant base substitution during replication (Cairns, 2006; Seaberg & van der Kooy, 2003; but see Sotiropoulou et al., 2008). "Immortal strand" retention may "apply to only a subset of stem cell lineages" (Neumüller & Knoblich, 2009), and epigenetic changes, such as an increase of methylation, may accumulate in "immortal strands" thereby compromising the efficacy of this "anti-mutation" adaptation (Genereux, 2009). But asymmetric division is a decidedly regulated process in some stem cells where it occurs, for example, in the GSCs of male *Drosophila* where the older "centriole is always in the centrosome that is … retained by the stem cell" (Gonzales, 2007). Hence, retaining "immortal strands" is not a mere coincidence and is presumably adapted to some function such as mutation prevention.

3.2 Finding and isolating stem cells

Putative stem cells are found by in situ hybridization with antibodies for specific antigens. For example, antigens for *Lgr5* gene products label LRCs in intestinal glands and hair follicles (Bussard et al., 2010b). Some markers are associated predominantly with malignant stem cells. For example, human breast cancer cells are CD44+CD24-/low (Al-Hajj et al., 2003), leukemic stem cells (LSCs) are CD34 positive CD38 negative (Bonnet & Dick, 1997), and colon cancer (O'Brien et al., 2007; Ricci-Vitiani et al., 2007) and human glioma cells are CD133 positive (Singh et al., 2004). Other markers change with malignant progression. For example, the "CD133+, epithelial-specific antigen-positive … population is increased in primary non-small cell lung cancer (NSCLC) compared with normal lung tissue and has higher tumorigenic potential in SCID mice and expression of genes involved in stemness, adhesion, motility, and drug efflux than the CD133− counterpart" (Bertolini et al., 2009). But problems arise over the antigen detected, the antibody used, the specificity of the antigen/antibody complex (see Lobo et al., 2007; Rao et al., 2010), and how closely tied the antigen is to a self-renewal signal pathway (Barker & Clevers, 2007).

Happily, some techniques accommodate multiple criteria allowing for "cross checking." Conspicuously, cytometric cell sorting allows researchers to combine multiple criteria for stem cells while providing living cells for further experimentation. With the help of fluorescence-activated cell sorting (FACS; Watt, 1998; Osborne, 2010), researchers can isolate presumptive OSCs, HSCs, CSCs, malHSCs, and putative malESCs in a "side population"

(SP) of cells able to reduce their load of supravitally absorbed dye (i.e., they exhibit Hoechst 33342 or Rhodamine 123 "effluxing"). Much like chemotherapeutic reagents, incorporated Hoechst 33342 and Rhodamine 123 are pumped out of (i.e., "effluxed" from) presumptive stem cells via the action of transporters (i.e., members of the ABC transmembrane protein family such as the ABCG2 transporter pump in mice) said to be uniquely over expressed in stem cells and embedded in their boundary lamella. Thus, presumptive stem cells have been isolated in SP fractions of cells from a host of normal organs, tissues, and cell populations: bone and dental tissues, cardiovascular tissue, endometrium (lining the uterus), endothelia (lining blood vessels), epidermis, gastrointestinal epithelium, mammary gland, neural tissue, pituitary and thyroid glands, and elsewhere (Welm et al., 2003; see Telford, 2010).

And some SP cells originating from cancers also pass the "gold standard assay" and form tumor-like nodules in minimum, low adhesion medium, while they produce histologically recognizable tumors in histo-compatible mouse strains such as immuno-incompetent nude mice, immuno-compromised non-obese diabetic (NOD), severe combined immunodeficient (SCID) mice, combined NOD/SCID mice, and more severely genetically compromised NOD/SCID mice. These SP cells also carry stem cell-relevant antigens and cell markers, for example, antigens associated with high plasticity (Sox2 and Oct4, but see Lengner et al., 2007), embryonic activities (stage-specific embryonic antigens [SSEA], Nanog, Sox4, Isl-1, and Pax6; see Konala et al., 2010), and specific histotypic markers (pituitary specific factor [Prop1]) alone and in combination (Garcia-Lavandeira et al., 2009).

A problem arises, however, about the size of a transplantable stem cell population identified operationally in the SP fraction. When does size exceed reasonable expectations for "a small subpopulation" conforming to traditional expectations for stem cells? Consider, for example, a "tumorigenic subpopulation with [melanoma] stem cell properties enriched in a CD20+ [SP] fraction [that] produces tumor-like non-adherent spheroids in culture with the plasticity of neural crest stem cells and a capacity for self-renewal" (Fang et al., 2005). A small percentage (<0.1%) of these cells are transplantable in NOD/SCID mice, but as much as 20% of "melanoma tumor stem cells" (MTSCs) positive for neural growth factor receptor CD271 (Boiko et al., 2010) give rise to tumors in more highly immune-compromised mice (i.e., NOD/SCID mice lacking the interleukin-2 gamma receptor, i.e., natural-killer cell activity; Quintana et al., 2008). This high percentage of melanoma cells able to transfer the tumor to these mice "suggests that either virtually every melanoma cell is a CSC because it can induce de novo tumors in xenograft assays irrespective of any known stem cell marker, or that melanoma is not hierarchically organized into subpopulations of tumorigenic and nontumorigenic cells and the CSC model does not apply" (Roesch et al., 2010).

3.3 Normal and malignant stem cells: Comparisons and contrasts

Stem cells sit on top of differentiation pyramids of cells (Reya et al. 2001). Hence, inevitable similarities appear in normal and malignant stem cells. "Indeed, in several tissues, normal stem cells and cancer stem cells (CSCs) have been identified using the same set of markers" (Dey & Rangaragan, 2010). For example, paired antigens are found in lung parenchyma and malignant adenocarcinoma of the lung (Kim et al., 2005) and in pancreatic acinar and pancreatic cancer cells (Hermann et al., 2007).

Some similarities are readily attributed to routine functions performed by normal and malignant cells. For example, cells of both types undergo mitotic cycling, periodically going through mitosis (M [chromosomal events prior to and accompanying cell division]) followed by a post-mitotic gap (G_1), a period of DNA synthesis (S), and a pre-mitotic gap

(G$_2$). And some similarities may be superficial (i.e., analogies instead of homologies). Conspicuously, "self-renewal" in stem cells may be a consequence of "transformation" or immortalization (Shay et al., 2001). Immortalized normal cells even become tumorigenic when introduced in immuno-compromised mice. For example, human B-lymphoblastoid cell lines immortalized by the Epstein-Barr virus become cancer-like in several ways: expressing telomerase (the ribonucleoprotein that elongates telomeres), exhibiting aneuploidy (an abnormal number of chromosomes), sustaining mutations in the cancer suppressing p53 gene, and failing to undergo apoptosis (Sugimoto et al., 2004). And immortalization is effected by a variety of devices that may be irrelevant to oncogenesis or over-determined: fusion with cancer cells, treatment with carcinogens, transfection with particular oncogenes such as myc, activation of normal cellular proto-oncogenes, transformation with Epstein-Barr virus, retrovirus-mediated oncogene transduction, human T-cell leukemia virus type 1 (HTLV-1) and simian virus 40 large T-antigen oncogene, human papillomavirus, etc.

On the other hand, some difference may be rationalized with the help of reasonable argument. For example, the difference between symmetric and asymmetric division may be reconciled if division in stem cells is facultative rather than constitutive and if the same cells that contribute to homeostasis via asymmetric division can support growth via symmetric division (Morrison & Kimble, 2006). In male rats, for example, differences in the mode of division depend on conditions. Large cells with outer membranes rippling with amoeba-like pseudopods (as opposed to cells with a smooth outline) are committed GSCs (aka gonocytes) that perform both asymmetric and symmetric division. Although male GSCs maintain a steady state population as spermatogonia in adults, the cells proliferate symmetrically and generate spermatogenic colonies when transplanted to infertile testes (Orwig et al., 2002). Thus, at least some stem cells would seem able to divide both asymmetrically and symmetrically.

Greater difficulty is encountered rationalizing differences in label-retaining cells (LRCs), namely, their presence among OSCs and CSCs versus their absence in HSCs and malHSCs (but see Wilson & Trumpp, 2006). Caveats aside, if HSCs and malHSCs are not LRCs, they cannot differentially segregate new and "immortal strands" of DNA during asymmetric division (Kiel et al., 2007). And other differences cannot be ignored. For example, while OSCs and (most) CSCs are confined to niches, HSCs and malHSCs circulate in peripheral blood (and umbilical blood, in the case of the fetus and newborn). Furthermore, unlike products of OSCs and CSCs, products of HSCs and malHSCs, and their dormant memory cells (see below) may regain self-renewal.

HSCs also exhibit far greater potential than OSCs and give rise to clones of hematopoietic proliferative precursors or progenitors (HPPs) with greater competences than transit-amplifying cells (TACs) produced by OSCs. In vivo, bone marrow derived HSCs known as stromal cells have a reputation for extraordinary "transmutation" to nerve and other non-hematopoietic cells, even if, in vitro, their range of transformations narrows to osteoblasts, chondrocytes, adipocytes, and possibly myoblasts (Prockop, 1997). Consequently, HSCs were once thought to be available for extensive "reprogramming" and multilineage differentiation compared to other stem cells. Prior to 2006 when induced pluri-potential stem cells (iPSCs) came along, HSCs were supposed to be the great hope of regenerative medicine (Trounson, 2009).

Reprogrammability is not open ended, however, and early hopes for HSCs' did not pan out despite their vast multi-potentiality. HSCs failed to exhibit pluripotency (the ability to

differentiate into tissues formed by all germ layers) when injected into blastocysts (Geiger et al., 1998) and failed to differentiate as cardiac myocytes when injected into damaged hearts (Murry et al., 2004). Some claims for HSCs' multipotency may have been exaggerated as a consequence of fusion with differentiated cells (Terada et al., 2002; Ying et al., 2002; Wagers & Weissman, 2004). The ability to fuse may be an interesting characteristic of HSCs and HPPs, but it is not especially promising as a method in regenerative medicine. Ultimately, instead of progress toward applications in regenerative medicine, "confusion looks set to continue" (Check, 2007).

In addition, significant differences abound among post-stem cell (non-self renewing) products in normal tissues and cancers. TACs and HPPs both divide symmetrically producing clones of bulk cells committed to determined pathways of terminal differentiation and disposal, but HPPs have vastly greater competences for differentiation than TACs. The products of CSCs and malHSCs also differ in their plasticity, with malHSCs sometimes called "primitive HSCs" because of the greater range of malignant phenotypes available to them.

Typically, the malignant phenotype "progresses" from dividing and invading cells destroying tissue locally to metastasizing cells repeating these processes at new sites. In the process, CSCs produce cancer transit amplifying cells (CTACs). The CTACs of less malignant cancers, such as teratocarcinomas, undergo terminal differentiation in any of a variety of directions. More generally, the "difference between cancer and normal tissue renewal is that in normal tissue renewal, the number of cells that are proliferating is essentially equal to the number of cells terminally differentiating (undergoing apoptosis), whereas in cancer the number of cells that are proliferating ([cancer] transit-amplifying cells) is greater than the number of cells that are entering terminal differentiation, because of maturation arrest of the cancer cells in the transit amplifying population" (Sell, 2008). In more malignant carcinomas, CSCs or CTACs pass through an epithelial-to-mesenchymal transition (EMT), become motile, and all the more malignant and metastatic (Prindull & Zipori, 2004). Likewise, malHSCs produce malignant HPPs (malHPPs) that not only display the malignant phenotype but are recruited to metastastatic sites from circulation. MalHPPs have also been accused of re-acquiring self-renewal with its consequent resistance to radiation and chemotherapy (Lapidot et al., 1994).

Disposal also takes place through different mechanisms in the products of different stem cells. In cellular apoptosis or caspase-dependent cell fragmentation, cell fragments known as apoptotic bodies are ingested and digested by neighboring cells (known as entosis) leaving healthy tissue behind. In tissue disposal or caspase-independent programmed cell death, aka autophagy, cytokines attract leukocytes and immune cells inducing an inflamed response and mass destruction. Other cellular disposal methods include phagocytosis by macrophages in localized centers (e.g., spleen, thymus) of effete cells marked by components of the complement and/or immune system, and the shedding of mature cells at topographically external surfaces.

Unlike normally produced TACs and HPPs, AMLs produce massive numbers of malHPPs that die before differentiating (Bonnet and Dick, 1997). In contrast, CTACs may have prolonged lifetimes as a consequence of delayed programmed cell death. "When baseline levels of autophagy are compared with many cancer cells and noncancerous cells from the same tissue, decreased autophagy is observed in many cancer cells … [C]ells within the center of the tumor, deprived of an adequate blood supply have upregulated autophagic flux to allow for survival in the hypoxic and low nutrient microenvironment … Many cancer

therapies considered over the last couple of years have been thus paradoxically aimed at either inducing or reducing levels of autophagy" (Demaria et al., 2010).

In sum, the closer one looks the harder it seems to harmonize stem cells. Even *bona fide* stem cells do not fall comfortably into a single category. Stem cells cannot be present in small and large numbers, divide infrequently and frequently, be both long-lived and short-lived and both capable of retaining "immortal strands" of DNA and not. Oncologists, like other scientists suffer from the tendency to lump phenomena together and to over-generalize, but lumping cells together under the "stem" umbrella does not illuminate the mysteries of cancer. Thus, the possibility of tracing cancers' stem cells' origins to a rudimentary stem cell must be abandoned and the search begin again elsewhere.

4. Are cell populations the roots of cancers' evolution?

Did cancers' stem cells evolve through mutual competition and selection in cell populations? The problem answering this question is that little is known about cell populations and virtually nothing about their evolution.

Cell populations are groups of cells sharing developmental and morphological characteristics. Cell populations are the constituents of tissues (i.e., epithelia, connective, blood and lymphatic, muscle, and nerve tissue), of parenchyma (i.e., major, conspicuous or characteristic cell type), and stroma (i.e., supporting the parenchyma) of organs (Baker, 1988; Hughes, 1989; Harris, 1999). Initially, "cell populations constituting multicellular organisms … [were] roughly classified, based on their kinetics, into three main groups," static, transit, and stem (Lajtha, 1979). This classification required amendment, since "transit" cells were derived from stem cells and did not, therefore, constitute a unique class, and other cell populations were not static, transit, or stem (e.g., the endothelium of vessels).

Table 1 is a new taxonomy for animal cell populations at homeostasis based on three dichotomous descending divisions: (1) Classes of attached or epithelial-like cell populations versus unattached or amoeba-like cell populations, (2) subclasses of steady state versus static cell populations, and (3) subsets of stem versus non-stem cell populations. Both stem and non-stem populations are found in three of the four subclasses, the exception being the attached, static state subclass containing only stem-like (reserve) cell populations. In addition, the subset of unattached, static, non-stem cell populations is partitioned into cell populations with stress-induced and developmentally produced dormancies.

4.1 Classes, subclasses and subsets of cell populations

Attached or epithelial-like cells are mounted on an extracellular membrane (e.g., the basal lamella of the epidermis) and share intimate contacts with each other in the form of intercellular and gap junctions or synaptic junctions. Nuclei are typically enclosed in a cytoplasm limited by a plasmalemma, but cells may also fuse in syncytia containing multiple (nondividing) nuclei. Cells in attached populations have limited plasticity or range of differentiation. Mono-potent cells differentiate into only one type of cell, and oligo-potent cells differentiate into a few related types of cells.

In contrast, unattached or amoeba-like cells are embedded or suspended in extracellular material and do not have intimate contacts with each other. Amoeba-like cells may have intercellular bridges (sex cells; see Shostak 1991) or be fused in plasmodia (*Physarum*) with mitotically active nuclei (as distinct from syncytia). Unattached amoeba-like cells also tend to be oligo-potent or multi-potent, having competence to differentiate into more than one cell type epitomized by germ cells.

Steady state cell populations produce as many cells by cell division as they lose through terminal differentiation and cell disposal. In contrast, static state cell populations do not produce new cells and lose cells primarily as a result of wear-and-tear, trauma, and aging.

Stem cell-supported populations are hierarchal containing different types of dividing cells, some of which (i.e., stem cells) are self-renewing and also give rise to clones of terminally differentiating cells. The populations may cycle at a constant rate, and be homogeneous, or they may cycle at different rates, move out of phase, and be heterogeneous.

In contrast, non-stem cell populations are non-hierarchal containing uniformly dormant cells or more or less identical cells that are both dividing and differentiated. Cells divide symmetrically in or out of phase. They are non-hierarchal, since they are more or less uniformly differentiated, although differentiation may proceed stochastically, regressively, or progressively across spatial and physiological gradients.

4.2 Specific categories of somatic cell populations

All adult somatic cell populations fall into eight categories (Table 1): (1) cache cells (CCs), (2) organ stem cells (OSCs), (3) reserve cells, (4) neoblasts, (5) stressed cells, (6) quiescent cells, (7) hematopoietic stem cells (HSCs), and (8) mesenchyme. Cache cells and neoblasts are primitive cells in the attached (epithelial) and unattached (amoeboid) categories, respectively. Other normal cells in these classes represent derived cells including germ cells placed in the HSC category. Neoplasm occurs and cancers develop in all but two of the categories, namely 4 and 5.

The origin of germ stem cells (GSCs) from amoeboid, neoblasts, and interstitial cells in invertebrates, and conspicuously from wandering cells in vertebrates relegates GSCs to the unattached cell line and places them in the HSC category. The amoeboid spermatozoon of nematodes makes the case plainly, and, like vertebrates' HSCs, embryonic GSCs invade and colonize ectopic sites (germinal ridges).

Because embryonic stem cells (ESCs) are not recognized in adult tissue, they do not appear in Table 1. Neoplasm typically attributed to malignant malESCs, however, is cited in categories 2, 7, and 8.

4.2.1 Cache cells (CC) and cancer cache cells (CCCs)

The parenchyma of glandular organs (e.g., liver) is typically comprised of CCs. The cells "appear mitotically equivalent" (Rhim et al., 1994) and uniformly differentiated (but see Alison, 1998; König et al., 2005). The population is non-hierarchal and steady state. Cells are mono-potent, committed to their specific cell type. Their state of differentiation may change stochastically or gradually. In the liver, for example, CC differentiation regresses as cells move centripetally on septa.

Cells are called "cache cells" because they constitute a "horde" of similar cells that can exceed normal rates of proliferation during regeneration the way a computer's "cache memory" promptly retrieves data (Shostak, 2006). Previously, parenchymal cells, such as hepatocytes were dubbed "expanding" cells (Leblond, 1972), because nearly all of them undergo cell division during regeneration (e.g., induced by partial hepatectomy), and the population's size expands virtually exponentially (Bucher & Swaffield, 1973). But at homeostasis the size of CC populations does not change, and the notion of expansion is inappropriate.

Attached, epithelial-like, mono- oligo-potent:

Steady state:

Non-stem, symmetrical division, differentiated, non-hierarchal:

(1) Cache cells (CCs) and cancer cache cells (CCCs)

CCs: superficially uniformly differentiated, mono-potent; CCCs: hepatoma carcinoma, angiosarcoma, (lymphangiosarcoma, or hemangiosarcoma), Kaposi sarcoma

Stem, asymmetrical division, hierarchal populations:

(2) Organ stem cells (OSCs), cancer stem cells (CSCs), and malignant embryonic stem cells (malESCs)

OSCs: self-renewing, homogeneous, produce TACs: symmetrically dividing, clonally committed, terminally differentiating, limited potency; CSCs: expanding, metastatic, produce CTACs: adenocarcinomas, non-small cell lung cancer (NSCLC); malESCs: heterogeneous tumors differentiate in embryo-like patterns (melanoma, glioblastoma)

Static state:

Stem-like, induced asymmetrical division, hierarchal:

(3) Reserve and reserve cell-derived cancer cells

Undifferentiated, arrested, retain ability to divide and differentiate, mono-potent; malignancies: rhabdomyosarcomas

Unattached, amoeba-like, oligo- multi-potent:

Steady state:

Non-Stem, symmetrical division, non-hierarchal:

(4) Neoblasts

Undifferentiated, cell division regulated by nutrition, multi-potent

Static state:

Non-Stem, stress (starvation) induced mitotic arrest, retain ability to divide, non-hierarchal:

(5) Stressed (regeneration or stockpile) cells

Undifferentiated, stress induced mitotic arrest, may resume mitosis when stress is lifted (i.e., animals fed)

Non-Stem, developmentally induced mitotic arrest, retain ability to divide, non-hierarchal:

(6) Quiescent cells and their derived cancer cells

Differentiated, developmentally induced mitotic arrest (e.g., Hayflick limit); may be irretrievably arrested and nil-potent (*C. elegans*) or resume division conditionally and oligo-potent (vertebrates); malignancies: fibrosarcoma, synovialsarcoma

Steady state

Stem, asymmetrical division, hierarchal:

(7) Hematopoietic stem cells (HSCs), malignant HSCs (malHSCs); malignant ESCs (malESCs), germ stem cells (GSCs) and malignant GSC (malGSCs)

HSCs: heterogeneous, produce hematopoietic proliferative precursor (HPPs) and memory cells, multi-potent; malHSCs (aka cancer initiating cells [CICs]), expanding metastatic malignant (malHPPs), leukemia, lymphomas; malESCs: small cell lung carcinoma; malGSCs: testicular and ovarian cancers

Static state

Stem-like, retain ability to divide, non-hierarchal:

(8) Mesenchyme (aka mesenchymal stem cells) and mesenchyme derived cancers (also malESCs)

Unifferentiated (fibroblast-like), arrested, oligo- multi-potent, malignancies: chondrosarcomas, osteosarcomas, malignant fibrous histiocytoma, and liposarcoma

Table 1. Classification of cell populations

The regeneration of CC populations would seem dependent on multiple controls. Regeneration in the liver, for example, tapers off when a normal mass is approximated irrespective of morphology, but a liver with its regenerative capacity exhausted by severe or chronic liver disease may yet regenerate as a function of proliferation by small stem-like oval cells in the intrahepatic bile ductules and (possibly) through the recruitment of extra-hepatic stem cells from bone marrow (König et al., 2005).

The mesothelium of the plural, pericardial, and peritoneal cavities, and the endothelium of vessels belong in the CC category, although endothelium is sometimes said to harbor stem cells (Potten et al., 1979). Endothelium may also consist of "mixed" CC and OSC-supported populations, and in glioblastoma, the presence of the same genomic alterations in a high percentage of endothelial cells and glioblastoma cells suggests that malignant neural cells transform into endothelium (Ricci-Vitiani et al., 2010; Wang et al., 2010) without cell fusion (Wurmser et al., 2004). The pancreatic parenchyma may also consist of "mixed" cell populations. Pancreatic islet cells divide symmetrically and thus qualify as CCs, although β pancreatic islet cells do not replace cells lost in type 1 diabetics (Dor et al., 2004). Pancreatic acini, on the other hand, harbor "multi-potent" stem cells with "a limited capacity for self renewal" (Weir & Bonner-Weir, 2004; Seaberg et al., 2004; Sangiorgi & Capecci, 2009; but see Brennand et al., 2007; Ku, 2008).

Polyploidy (i.e., abnormal multiples of the chromosome number) and binuclearity (i.e., the presence of two nuclei in a cell) are widespread among CCs. These conditions do not represent adaptations to streamlining regeneration, since smaller mononuclear diploid cells provide most new cells during regeneration (Sigal et al., 1999). Polyploidy and binuclearity may represent accommodations to increasing metabolic demands, since cells with these traits accumulate with age, chronic stress, and oxidative injury (Goria et al., 2001). But nuclei of binuclear cells may also be evidence of degenerate change. When two nuclei fuse and divide symmetrically, they produce tetraploid cells (Guidotti et al., 2003), and "ploidy reversal" or "reductive mitoses" occurring despite bipolar spindles results in chromosomal imbalance and aneuploidy (Duncan et al., 2010) conducive of cancer (Ganem et al., 2009).

CC populations may spawn symmetrically dividing cancer cache cells (CCCs). Endothelial CCCs, for example, are probably the source of angiosarcomas (lymphangiosarcoma, or hemangiosarcoma), and the spindle cells of Kaposi sarcoma may also be CCCs. Angiosarcomas and glioblastomas would seem to be composed of CCCs, but CCCs may become CSCs in "mixed" tumors consisting of CCC-like differentiated cells and undifferentiated CSC-like cells (e.g., polycythemia [myeloproliferative neoplasms]; Jepson, 1969).

Malignant hepatoma cells of hepatocellular carcinomas are archetypal CCCs. They divide symmetrically, rapidly and are sensitive to chemo and radiation therapy. Irradiated cells may be arrested at the G_2/mitosis checkpoint if the DNA damage caused by radiation exceeds a threshold of two chromatid breaks or "a few" double-strand breaks (Ishikawa et al., 2010). Surprisingly, rat malignant hepatoma cells are oncogenic or not depending on their site of introduction and age of a host. Possibly, instead of a homogeneous CCC population, a heterogeneous population includes subsets able or not to establish themselves in different circumstances (McCullough et al., 1998).

4.2.2 Organ stem cells (OSCs) and cancer stem cells (CSCs)
OSCs exhibit self-renewal by asymmetric division. They are the classic label-retaining cells (LRC) thought to divide infrequently and frequently retain "immortal DNA" strands. In

contrast the TACs produced by OSCs divide rapidly and symmetrically producing clones of cells, typically with limited potency.

OCSs occupy distinct niches where they undergo self-renewal (Li & Xie, 2005). Some niches are conspicuous such as the corneal limbus basal layer (Sun et al., 2010), the bulge of hair follicles (Clayton et al., 2007; Hsu et al., 2011), and the ends of intestinal glands between enteroendocrineocytes (Potten & Loeffler, 1990; Barker & Clevers, 2007). But some niches, such as the subventricular zone of the cerebral cortex and spinal cord (Lois & Alvarez-Buylla, 1993; Weiss et al., 1996; Merkle et al., 2004; Maric et al., 2007; Doetsch et al., 2009) are only identified loosely as areas of asymmetric division (Lajtha, 1979; Tumbar et al., 2004) and might not truly qualify as niches, since the "simple location of stem cells is not sufficient to define a niche. The niche must have both anatomic and functional dimensions, specifically enabling stem cells to reproduce or self-renew" (Scadden, 2006).

In the mammalian epidermis, self-renewal is constrained by the differential expression of β-1 integrins and binding to the extracellular matrix (Lavker & Sun, 2000). The niche determines if TACs form hair follicles, hair, and sebaceous glands (Hsu et al., 2011) or if blocks of cells moving outward through the epidermis toward the surface synthesize a variety of keratins and finally differentiate as disposable squames (Blanpain & Fuchs, 2006). Epidermal cells occupying other niches produce fingernails, toenails, claws, and hooves. In the small intestine, basal glandular niches (Barker & Clevers, 2007; Fellous et al., 2009) produce TACs that divide and differentiate. Absorptive, dome (M), and goblet cells (Lelouard et al., 2001) move outward and are disposed of en masse at the intestinal surface. Parietal and chief cells, enteroendocrinocytes, and exocrinocytes stay in the gland until they are disposed of individually.

Some astrocyte stem cells in the central nervous system (CNS) exhibit moderate oligopotency, since the products of their division differentiate as disposable neurons and glial cells (Quian et al., 2000; Doetsch, 2003; Walton et al., 2006). The CNS is derived from neuralectoderm, and, hence, from epithelium, but neuro/glioblasts produced by astrocyte stem cells are motile and amoeba-like, and the ependymal home of astrocytes (Weiss et al., 1996) lacks a basal lamina and therefore does not qualify as an epithelium. Neuro/glioblasts, thus have taken on amoeboid characteristics after de-epithelializing.

The relationship of OSCs to CSCs is ambiguous. Some solid tumors supported by CSCs share antigens with OSCs, and a stem subset among otherwise non-tumorigenic cells may express tumorigenicity (Bonnet & Dick, 1997; Al-Hajj et al., 2002; Hermann et al., 2007). CSCs do not necessarily arise in the same niches as those occupied by OSCs. For example, basal cell carcinoma arises in inter-follicular epidermis rather than the hair follicle's bulge (Youssef et al., 2010). On the other hand, malignant stem cells, such as those of non-small cell lung cancer (NSCLC), an adenocarcinoma, may be derived in situ from bronchioalveolar OSCs following malignant transformation, and the CSCs of breast and colon cancers share affinities with OSCs.

4.2.3 Reserve cells and cancers derived from reserve cells

Reserve cells are undifferentiated dormant cells within a differentiated (typically, but not exclusively static) parenchyma derived from attached cells. Reserve cells include astrocytes (Rice et al., 2003; Martens et al., 2002) and pancreatic acinar cells (Sangiorgi & Capecci, 2009), but satellite cells (also known as quiescent myoblasts) in skeletal muscle are archetypal (Hawke & Garry, 2001). The satellite/skeletal muscle framework suggests that satellite cells are mammalian skeletal muscles stem cells held in mitotic abeyance.

Satellite cells reside within or beneath the external lamina of muscle fibers (in the sublaminal space or zone between the lamina and the sarcolemma of the muscle fiber) and are distributed evenly along the length of muscle fibers (with the exception of the neuromuscular junction). The sites occupied by satellite cells constitute a diffuse niche adapted to permit regeneration over the length of muscle fibers. During skeletal muscle regeneration, satellite cells become self-renewing, albeit briefly (Schultz, 1996) via asymmetric division. The stem cells exhibit differential "immortal DNA" strand retention, and the precursors of muscle fuse with sarcomeres and differentiate as skeletal muscle (Tajbakhsh et al., 2009).

Reserve cells seem to have left the division cycle in the G_1 post-mitotic gap. Following trauma, the proportions of satellite cells in S and G_2 increase rather than drop-off demonstrating that cells have moved through the cycle (or that other cells have undergone apoptosis disproportionately; Relaix et al, 2006).

Reserve cells are frequent suspects in the cancer lineup. Rhabdomyoblasts, or embryonic and fetal skeletal muscle cells appear in benign rhabdomyoma, in malignant rhabdosarcoma, embryonic, alveolar, and adult rhabdomyosarcomas. The precise etiology of rhabdomyoblasts is uncertain, but satellite cells may be their precursors (Merlino & Helman, 1999; Mercer et al., 2006).

4.2.4 Neoblasts

"Neoblast" is the generic term for dividing amoeboid cells in many well-fed, sponges, cnidarians, flatworms, and other protostomes. Neoblasts exhibit multi-potentiality during steady-state homeostasis, during regeneration, and somatic asexual reproduction, differentiating into a wide range of cells in the animal's body. Hence, neoblasts are also called "stem cells" in the sense that they "branch" out and differentiate into a variety of non-dividing cells, although they do not fulfill the additional stem-cell criterion of occupying a niche and representing a small slowly dividing part of a proliferative population. A distinguishing characteristic of neoblasts in flatworms and elsewhere is that cell division is down regulated by stress such as that brought on by starvation (Newmark & Alvarado, 2000; Reddien & Alvarado, 2004).

No malignant growths are attributed to neoblasts. Cell division in neoblasts seems to be held in check by homologues of the human p53 cancer suppressor gene which "functions in [planarian] stem cell proliferation control and self-renewal" (Pearson & Alvarado, 2010).

4.2.5 Stressed cells

Stressed cells (aka regeneration or stockpile cells) in invertebrates are derived from neoblasts and similar cells after entering mitotic arrest typically induced by starvation (Hong et al., 1998). In flatworms, the rate of cell division in neoblasts declines to a "basic level" as a result of starvation (Nimeth et al., 2004). These stressed cells are arrested at the G_2 stage, presumably as an adaptation for a rapid return to mitosis. G_2 arrested cells disappear following the resumption of feeding. The resulting highly plastic neoblasts then resume differentiating along multiple paths. In other animals, stressed cells may abandon the division cycle in G_1. When arrest is persistent, these cells are identified as G_0 or G_1/G_0 cells. No malignancies are attributed directly to stressed cells, although malignant cells may be "stressed."

In vertebrates, mitotically arrested cancer cells suffering from energy deficiencies due to a carbohydrate deficit might be considered stressed cells. Cancers acquire their energy largely

by glycolysis. Indeed, cancers' demand for glucose, known as cancers' "sweet tooth," and the enhancement of glycolysis, known as the Warburg effect after Otto Warburg who discovered it in 1924, are dose dependent and correlated with the aggressivity of the malignancy in vivo (Elstrom et al., 2004). The Warburg effect leads to the excess production of lactate that induces several oncogenes, causes an acid environment protecting cancer cells from the immune system, and allows pyruvate to scavenge endemic hyperoxides. At the same time, reduced cofactors remove free radicals and relieve high oxidative stress created meeting demands of rapid cell division (Kim et al., 2009). The Warburg effect also explains why tumors light up in positron emission tomography (PET) with a glucose radioisotope (Garber, 2004) and suggests that cancers might be selectively starved with low carbohydrate, high fat or insulin-induced hypoglycemia/lactate supplemented therapeutic diets.

Stress in mammals also triggers immuno-suppression that can be tumorigenic rather than therapeutic. For example, indirect deleterious effects of stress promote tumor development in rodents and human beings. Tumorigenesis under stress seems to result from immune suppression of natural killer cell activity (Ben-Eliyahu et al., 1999). For example, oxidative stress in myeloid cells makes them capable of inhibiting T-cell proliferation. The presence of oxidatively stressed cells "in a premetastatic niche … [may] help incoming tumor cells [i.e., CSCs] survive by inducing local immune suppression via inhibition of effector immune cells and by helping to evade immune system control, thus promoting metastasis growth" (Kusmartsev et al., 2008).

4.2.6 Quiescent cells and cancers derived from quiescent cells

Quiescent cells become mitotically dormant in the course of development (rather than as a consequence of stress). They are widespread in invertebrate adults. For example, in *C. elegans*, after adding cells throughout four larval stages, the hermaphrodite adult winds up with 959 quiescent somatic nuclei (1031 in males) arrested in G_0/G_1 (van den Heuvel, 2005).

In vertebrates, quiescent cells are represented conspicuously by fibroblasts (aka fibrocytes). Arrested in G_1, fibroblasts comprise numerous non-hierarchal, static state cell populations forming the bulk of stroma in organs including loose and dense, regular and irregular connective tissues. Osteocytes, chondrocytes, and possibly cardiac myocytes are also quiescent cells (Grounds et al., 2002).

Remarkably, although fibroblasts are not ordinarily dividing, they support division in other cells. An underlying layer of irradiated, non-multiplying "feeder" fibroblasts in vitro sustains cell division in other cells (e.g., embryonic stem cells, epithelial, and cancer cells). "Feeder" fibroblasts are employed to "condition" tissue culture media thereby promoting cell division and aiding the establishment and upkeep of fragile cell lines (Puck et al., 1956).

Fibroblasts can be provoked into division. They divide in the vicinity of wounds, in the uterine stroma during pregnancy, and in the breast during lactation. Dividing fibroblasts tend to remain fibroblasts although fibroblasts may be oligo-potent and differentiate into fat cells. And perichondral and periosteal fibroblasts also differentiate into cartilage and bone cells.

Freshly explanted fibroblasts in tissue culture perform a large but limited number of divisions (e.g., 50–70) after which the cells enter a period of "mitotic quiescence" that may last months but is eventually followed by cell death. Known as the Hayflick limit after Leonard Hayflick who discovered it in the early 1960s, the number of divisions performed by freshly explanted fibroblasts in vitro is inversely proportional to the age of the organism from which the fibroblasts were taken (Hayflick & Moorhead, 1961; Kill & Shall, 1990).

Possibly, telomeric shortening is the "replicometer" determining cells' Hayflick limit and mortality (Hayflick, 2000). The alleged "immortality" of transformed and cancer cells in vitro may be due to the over expression of telomerase and consequent maintenance of telomeres (Chan & Blackburn, 2002; Hackett & Creider, 2002; Shay et al., 2001).

Fibroblasts produce benign leiomyomas (aka fibroids), malignant fibrosarcoma, and synovialsarcoma. Fibroblasts are not otherwise prone to malignancy (but see mesenchyme below).

4.2.7 Hematopoietic stem cells (HSCs), malignant HSCs (malHSCs), germ stem cells (GSCs), and malignant GSC (malGSCs); malESCs (see 2 and 8)

HSCs are the root stem cells of blood and lymphatic cells and all their derivatives both in circulation and sequestered in connective tissue. HSCs also sprout branches virtually everywhere: osteoclasts in bone, microglia in the central nervous system, dendritic cells in epithelia, and macrophages (e.g., histiocytes and dust cells) in lungs and elsewhere.

Human HSCs are typically rhodamine 123 low and CD34 positive. HSCs, like their malignant counterpart, malHSCs, or "primitive HSCs" are typically heterogeneous with respect to rates of self-renewal and differentiation (Uchida et al., 1996; Hope et al., 2004). Cells range from rarely dividing stem-like cells to those on the verge of committed HPPs (Osawa et al., 1996).

HSCs are highly multi-potent and HPPs widely competent, differentiating across a wide range of cell types. Even after reserving the title HSCs "for cells already committed to a hematopoietic phenotype" (Herzog et al., 2003), HSCs include common myeloid progenitors (CMPs), similar to cells in *Drosophila* that provide endothelial cells lining vessels in addition to blood cells (Owusu-Ansah & Banerjee, 2009), highly plastic bone marrow-derived stem cells (BMDSCs or BMSCs), and marrow stromal cells (MSCs aka mesenchymal stem cells) that produce clones differentiating into fat, cartilage, and bone (see Kode & Tanavde, 2010). HPPs include multi-potent adult progenitor cells (MAPCs), and prolific myelogenous blast cells that give rise to the multitude of circulating and fixed blood and lymphatic cells. And while the small lymphocyte seems genuinely non-dividing (Bekkum et al., 1971), medium and large proliferative lymphocytes in lymphopoietic organs, germinal zones, and nodules remain in contention for dividing T or B lymphocytes as well as cells playing a host of roles in immunity.

HPPs can also become dormant "memory cells" (members of the B lymphocyte domain) that resume proliferation in response to unique antigens and growth factors (Ohta et al., 1998). Memory cells are way stations responsible for the secondary antibody response characteristic of acquired immunity and may function as "first responders" to new antigens.

The plasticity of HSCs and malHSCs suggests that they are accessible to extensive reprogramming and expansion of potential in the process of forming clones. Reprogramming, if that is what it is, may also occur in malHPPs. For example, BMDSCs pass through a "metaplasia/dysplasia/carcinoma progression" into adenocarcinoma of the stomachs of C57BL/6 mice chronically infected with *Helicobacter pylori* (Houghton et al., 2004). Moreover, BMDSCs form stromal myofibroblasts in esophageal adenocarcinoma including epithelial tumor cells and endothelial cells (possibly) following fusion with host cells (Hutchinson et al., 2010).

The production of malHSCs may also be determined by conditions rather than an inherent commitment to this particular fate. For example, "primitive" HSCs that become the malHSCs of leukemia/lymphoma stem cells (LSCs) over express the cancer inducing *bcl-2*

oncogene in the presence of serum containing the KIT ligand (also known as the steel factor cytokine or stem cell factor [SCF]) and undergo stimulated cell division at the onset of malignant differentiation (Domenet al., 1998; Domen & Weissman, 2000).

MalHSCs or LSCs also seem to be members of a heterogeneous population of cells differing in rates of self-renewal and degrees of commitment (e.g., in AML; Hope et al., 2004). LSCs seem to be common, since "more than 10% of cells in many mouse leukemia and lymphomas are transplantable" (Adams et al., 2007). In fact, AML cells in mice are easily transplanted to nonirradiated histocompatible (congenic) recipient mice (Kelly et al., 2007; Adams & Strasser, 2008) leaving the impression that bulk AML cells rather than stem cells as such are capable of propagating the malignancy.

The germ line fits the HSC mold. The adult male germ line beyond dormant spermatogonia is easily placed in this category of stem cells. Even dormant spermatogonia (Clermont, 1962) can be placed in the stem-cell category allowing that they mimic reserve cells. And the adult female germ line of mammals, once thought to be static, is now conceded to be stem. GSCs in the ovarian surface epithelium (OSE or germinal epithelium) produce primary follicles in vitro and in vivo while in contact with underlying connective tissue (Johnson et al., 2004; Bukovsky et al., 2005).

Germ line niches such as the basal compartment of seminiferous tubules (Lin, 1998; De Rooij & Grootegoed, 1998) may exert "extrinsic" influences on asymmetric divisions, but "intrinsic" cellular influences also affect the geometry of chromosomal delivery and the "unequal distribution of key regulators" (Kim & Hirth, 2009). In the *Drosophila* ovary, the position of oogonia near the end of terminal filaments seems to depend on the expression of the *piwi* gene that suppresses GSC differentiation while promoting self-renewal (Lin, 1997). Further down the filament, the oriented asymmetrical division of GSCs creates the cystoblast or germ-line cyst that gives rise to "assembly line organization, with each egg chamber representing a differentiated stem cell product whose position along the ovariole corresponds to its birth order" (Cox et al., 1998).

Remarkably, in *Drosophila*, asymmetric division in mutant GSCs takes place in the absence of the centrosome. "[C]entrosoms are not required for the proper orientation of the spindle relative to the … niche in female GSCs," but centriole orientation is essential for embryogenesis (Stevens et al., 2007), and spindle mis-orientation consequent to mutations may contribute to tumorigenesis. The activities of "tumour suppressors, *lgl, dig* and *scrib,* in controlling the asymmetric segregation of cell-fate determinants in larval neuroblast … [suggest] that impaired cell-fate determination itself could cause tumour growth" (Gonzalez, 2007).

MalGSCs are the presumptive cause of malignant testicular and ovarian cancers (Lin, 1997). Evidence linking malGSCs to GSCs is weak, but the nuclei of spermatogonia bear "cancer/testis" antigens (e.g., Brdt, SSX, NY-ESO-1, members of the melanoma antigen and SPANX families; MacLean & Wilkinson, 2005). (For malESCs see 4.2.2 and 4.2.8.)

4.2.8 Mesenchyme (aka mesenchymal stem cells [MSCs]) and cancers derived from mesenchyme

Mesenchyme is defined classically as the highly hydrated connective tissue of embryos (Shostak, 1991), but the drier adult connective tissue of bone, skeletal muscle, dermis, and heart are often said to contain mesenchyme (see Kode & Tanavde, 2010). The appellation "mesenchyme" is also attached to pericytes, contractile cells sharing the basal lamella with endothelium in capillaries and small venules. In addition, MSCs are frequently equated with

HSC-derivatives, marrow stromal cells (also MSCs), BMDSCs, and MAPCs. In effect, "mesenchyme" in adults is a synonym for a subset of generally quiescent fibroblasts readily mobilized for mitosis by growth factors. Mesenchymal cells are not known to be self-renewing and are not confined to a recognized niche, but they may otherwise resemble reserve fibroblasts. Alternatively, mesenchyme may be compared to a normally slowly dividing CC-like population but especially active in regeneration.

Mesenchyme's relationship to embryonic connective tissue must not be taken too literally or dismissed too lightly. Wnt genes link malignant mesenchyme to embryonic signal pathways. In malignant fibrous histiocytoma (aka high-grade undifferentiated pleomorphic sarcoma) expressing the DKK1 gene, the gene's protein, Dkk1, is an inhibitor of the Wnt developmental program. Inhibiting Wnt2 signaling in human MSCs or their progenitor cell products transforms them into malignant fibrous histiocytoma-like tumor cells following injection into immuno-compromised mice. Amazingly, reestablishing Wnt signaling in malignant fibrous histiocytoma returns the cells to their normal connective tissue status (Matushansky et al., 2007). Regrettably, Dkk1 does not perform the same trick in carcinomas.

Mesenchyme may also be a source of malESCs responsible for malignancies of soft tissue, in particular, following the malignant transformation of perivascular "mesenchymal" cells (Iwasake et al., 1987). Malignant chondrosarcomas and osteosarcomas may also have mesenchymal etiologies as may malignant fibrous histiocytoma and liposarcoma.

5. Evolution of normal and cancer cell populations

"Chance and necessity" (Monod, 1971) are the motors that drive evolution over the rocky road of Darwinian competition and selection. Multicellular animals have been on that road a long time and chance and necessity have had ample opportunity to work their magic on the tissues and cancers of animals. Epithelial cell populations would have the most ancient roots if attached cells evolved from biofilms and biomats (recently reassigned to the pre-Phanerozoic; Arp et al., 2001; Bengston et al., 2009). Newly discovered fossils of epithelial-like organisms clock in at 2.1 billion years before the present (El Albani et al., 2010). Amoeboid cells have ancient roots too if not quite as ancient as epithelia. Acritarchs associated with marine algae suggest that unicellular eukaryotes were around somewhere between the late Paleoproterozoic and Early Mesoproterozoic epochs, 1.6–1.3 billion years before the present (Knoll et al., 2006).

Competition between these life forms inevitably drove them into conflict, and a form of conflict resolution known as "escape toward" would have driven epithelia and amoeba into symbiotic relationships. Presumably, somewhere, some time, or, more likely, in many places and many times symbiotic relationships were attempted and an occasional one proved successful. Evolution's creative powers were then unleashed especially when "Life got big" (Narbonne, 2011) in the wake of fluctuating levels of free oxygen in the post-glacial early Ediacara (Yuan et al., 2011).

Today, the placozoan, *Trichoplax adhaerens* (Grell & Ruthmann, 1991) may be the last surviving purely epithelial metazoan, while vast numbers of amoeboid organisms testify to the continued viability of the amoeboid way of life. Competition and selection in epithelial/amoeba symbiotic organisms, however, proved more innovative and inventive, and led to the enormous diversity of tissues and organs found across the multicellular animal kingdom.

5.1 Evolution of cell populations and tumors with epithelial lineages

The evolution of epithelial-derived cell populations turns out to track increasingly sophisticated controls over cell division during the production of increasingly complex tissues. Solid tumors compete with epithelia and their derivatives largely by defeating controls over cell division while accommodating to tissue complexity.

The origins of an epidermis can be found in freshwater sponges (Demospongiae, Haplosclerida). Surface pinacocytes form an epithelium exhibiting close intercellular junctions that resist permeability and the diffusion of small-molecules while offering high transepithelial electrical resistance and a transepithelial potential. Pinacocytes retain these properties during regeneration and asexual reproduction and are not transformed into other types of cells (Adams et al., 2010).

In Hydra (Cnidaria, Hydrozoa), the epithelial epidermis and gastrodermis are composed of cache-like cell populations. In the epidermis, potency is limited to surface epithelium, battery cells, and possibly nerve and gland cells in the foot, while in the gastrodermis, potency is limited to digestive cells and possibly some digestive gland cells. The rate of cell division in these epithelia is a function of the availability of food (Shostak, 1979, 1982). Sustenance levels of feeding support the production of cells in sufficient quantities for maintenance (homeostasis) and regeneration. Feeding above sustenance levels supports growth, and further feeding supports asexual reproduction as well (Campbell, 1967a, 1967b; Shostak, 1968, 1974). Restraints on the growth of epithelia appear in some anthozoans (e.g., anemones), however, where body size and asexual reproduction are constrained (Shick & Hoffmann, 1980).

Regulation of growth increases in Platyhelminthese and Aschelminthes. Flatworms have a quiescent cellular epidermis (Rieger et al., 1991), and in adult round worms, with the exception of smaller species that retain a small number of cells, the subcuticular "epidermis" is a syncytium with quiescent nuclei plus a row of quiescent lateral line seam cells (Wright, 1991). The mere presence of multiple nuclei within a unified cytoplasm is not the explanation for mitotic dormancy, since nuclei in plasmodia such as those of insect eggs and the true slime mold, *Physarum*, divide abundantly. Rather, the absence of mitosis in syncytia would seem an adaptation for inhibiting growth.

Growth is also constrained internally as an accommodation to an unyielding integument or exoskeleton in animals where complexity militates against removing excess cells via asexual reproduction. The regulation of growth within the organism would also seem to have been a prerequisite for the evolution of complex internal organs (Extavour et al., 2005), and curbs on cell division seem to have ratcheted up with the complexity of parenchymal differentiation. In contemporary vertebrates, cells that have left their niches in embryos, such as sensory and motor neurons and skeletal muscle do not divide at all. Mitosis seems to have been curbed entirely in the course of evolution of highly differentiated cell populations where growth would be disruptive.

Other tissues adopted the steady state to meet size constraints without sacrificing the flexibility inherent in cellular replacement. CC populations epitomize steady-state cell populations, losing and gaining cells at the same rate in dynamic equilibrium. OSC populations then branched off CC populations when cell division was further restricted in a self-renewing population separated from the bulk of dividing TACs (Stanger et al., 2007). In OSC-supported populations, asymmetric cell division is confined to cells that remain in their niche following division. Reserve (satellite) cells evolved from OSCs by the further restriction of cell division to the point of arrest in G_1 "until needed."

Epithelial-derived tissues seem to have invested heavily over the course of their evolution in preventing oncogenic mutations. Thus, "the G_2/M checkpoint is invariably activated in cancer cells in response to DNA damage" (Wang et al., 2009). In G_2 arrested cells, entry to mitosis is blocked when Cdc25 phosphatases fail to remove the inhibitory phosphorylation of (inactivated) complexes of mitotic CDK, Cdc2 (aka Cdk1) and B-type cyclins. Moreover, the chief regulator of the G_1/S checkpoint is the tumor-suppressor p53 gene whose products also prevent the expression of NANOG and other embryonic stem cell factors associated with malignancy (Zbinden et al., 2010). The widespread retention of the "immortal strand" of DNA by OSCs and satellite cells would also seem an anti-mutation adaptation. The presence of label-retaining cells (LRCs) in breast and intestinal cancers (Trosko, 2006; Bussard et al., 2010b; Barker et al., 2008) suggests that these tumors' CSCs are derived from OSCs.

On the other hand, the cells of solid tumors seem to have devised mechanisms for competing successfully with the cells of normal solid organs. CCCs override the rules governing steady state dynamics in CC populations, and CSCs may have branched off OSCs by violating the terms of stem-cell regulation. "Mixed" cancers containing stem and non-stem cells (e.g., pancreatic cancer and myeloproliferative neoplasm) suggest that CCCs may also step-up to CSCs with increased malignancy. Thus, CCCs are equipped with two deadly weapons, the step-up to CSC and the epithelial-to-mesenchymal transitions (EMT) (Prindull & Zipori, 2004). With these weapons, tumor cell populations not only undermine the restraints imposed by cell-to-cell communication, but they escape the limits imposed by asymmetric division. Malignant cells increase in number, break out of their niche, and overpower normal defenses (Powell et al., 2010; Quyn et al., 2010).

Some solid tumors seem to begin as pure accidents. For example, cancers develop following "chromosome missegregation" of "lagging chromosomes" in damaged aneuploid hepatocytes (Ganem et al., 2009). And other epithelial cancers may be initiated, promoted, or progress through the accumulation of breaks, translocations, and errors of replication that prevent tumor suppressor genes from completing DNA repair, create aberrant products in signaling pathways, or permit the notorious EMT (see Ansieau et al., 2008). Genetic and epigenetic changes in some solid tumors suspend normal terminal differentiation and disposal, turning rapidly dividing TACs into malignant CACS. These malignancies are hotly pursued under the rubric of targeted therapy (Gilbert & Ross, 2009). For example, the Wnt/β-catenin, Hedgehog, and Notch signal transduction pathways of cell division are also pathways of differentiation and offer especially vulnerable points for therapeutic attack (Taipale & Beachy, 2001). In addition, these pathways are associated with tumor suppressors, such as PTEN (Stambolic et al., 1998) suggesting still other opportunities for therapeutic intervention.

5.2 Evolution of cell populations, leukemia, lymphomas, germ, and soft tissue cancers with amoeboid lineages

Amoeba-like cells are the obvious choice for ancestors of neoblasts, for unattached cells, and for freely moving cells including germ-line cells in multicellular animals. Contemporary amoebas even behave much like neoblasts and like scavenger blood cells in today's multicellular animals. "Interestingly … environmental cues such as temperature, starvation, and high population are potent inducers of autophagy in yeast, *Dictyostelium* and mammals … [as well as] dauer formation in *C. elegans*" (see below; Meléndez & Levine, 2009). Presumably, stress provokes ancient mechanisms in these cells' adaptive repertoire including the suspension of cell division.

Many amoebas cease dividing following starvation but resume cell division after turning to cannibalism. Similarly, large amoeboid cells or archeocytes in sponges (Porifera) acquire reserves by cannibalizing adjacent trophocytes in response to seasonal adversity and produce an encapsulated gemmule. When growth conditions return, the gemmule "hatches." Cells stream through the capsule's micropyle and commence cell division and morphogenesis.

Amoebas also exhibit multi-potentiality. For example, amoebas of the cellular slime mold (aka social amoeba), *Dictyostellium discoideum,* attracted by cyclic adenosine monophosphate (cAMP) to its source, congregate and differentiate into distinctively contrasting cells of slug and fruiting body (see Bonner, 1988; Margulis et al., 1990). Likewise, in freshwater sponges, multi-potential amoebocytes emerging from reduction bodies differentiate as choanocytes as well as various types of amoebocytes (Bisbee et al., 1989). In general, sponge amoeboid cells contribute to growth, maintenance, asexual reproduction, and regeneration by generating a variety of cells: fiber cells or desmocytes, muscle or myocytes, spongin-producing sponbioblasts, food-containing trophocytes, pigmented chromocytes, large archaeocytes, gland, and germ cells (Hanson, 1977).

In Cnidaria, amoeboid cells or interstitial cells produce as many as seven types of cnidocysts (average 3 per species; Shostak & Kolluri, 1995) as well as sensory and motor neurons, several types of gland cells (Hwang et al., 2007), and germ cells (Littlefield, 1985, 1991). Amoeboid cells also fill regression bodies in response to adversity, undergo multi-potent differentiation during regeneration (Shostak, 2005), and participate in asexual reproduction through budding, regenerative fragmentation, strobilation, and fission (Shostak, 1993).

In well-fed flatworms, multi-potential neoblasts proliferate and differentiate (Newmark & Alvarado, 2000). By replacing effete cells, neoblasts maintain specialized organs, the epidermis enclosing the animal, the gastrodermis lining its gut, and the "fixed" parenchymal cells between these epithelial layers. Neoblasts also aid in remodeling the animal during regeneration and reconstituting it during asexual reproduction (Pellettieri et al., 2010). In starving animals, neoblasts become dormant stressed cells but return to the neoblast status upon the resumption of feeding.

Likewise, larvae of the celebrated round worm, *C. elegans,* respond to stress by "conditional cell cycle arrest" (Hong et al., 1998). Thus stressed newly hatched, L1 larvae cease developing and enter the dauer diapause. Stressed cells remain in mitotic suspension indefinitely, prolonging the life of the larva (hence *dauer*), but, when conditions permit, the cells return to mitotic cycling, and development resumes (Meléndez & Levine, 2009) along determined lines of differentiation (Sulston et al., 1983).

Amoeba-like cells left a long evolutionary line of descendants in vertebrates from connective tissue to germ with blood and lymph cells prominently in the middle. HSCs and malHSCs are enormously plastic and spawn a variety of blood, lymphatic, and connective tissue cell types, normal and malignant. Their version of "stemness" has unique features. HSCs and malHSCs appear outside their niche in circulation. Recruitment or self-seeding is also characteristic of these stem cell. Thus HSCs repopulate organs (e.g., bone marrow, lymph nodes, and thymus) depleted by disease or radiation, while the arrival of circulating malHSCs (aka cancer initiating cells [CICs]) at sites of metastasis and the further recruitment of circulating malHSCs or malHPPs would seem at least partially responsible for the growth of leukemia/lymphomas (Zon, 2008). Recruitment might also be a point of attack for intervention. Leukemia/lymphomas might be kept from growth and brought back to the steady state by recreating the "environmental guidance" that prevents recruitment (McCulloch, 1983).

Fibroblasts of connective tissue seem to have adopted quiescence as a way of restraining growth, although cell division may still be an option as it is in so-called mesenchyme. Benign growths of fibroblasts do not compare with malignant sarcomas presumably of mesenchymal origin.

Both male and female germ lines clearly evolved from amoeboid cells as witness the extensive intercellular bridges present in pre-germ cells (see Shostak, 1991), while the "pseudopods" on the outer lamellae of male gonocytes would seem perfect reminiscences of amoeba. On the other hand, the epithelial-like zona pellucida (i.e., an extracellular membrane) surrounding mature mammalian eggs would seem a harbinger of epithelialization of the future blastocyst.

5.3 Evolution of cell populations with malignant embryonic cell lineages

Because the rates of cell division in some tumors, leukemia, and lymphomas actually approach exponential growth (see Shibata & Kern, 2007-8), cancers are sometimes said to represent the release of arrested embryonic cells (Sell, 2008) or a transformation of adult cells to an embryonic state (Weinberg, 1996). But high rates of cell division are also found in normal adult OSCs. Mouse intestinal OSCs, for example, divide once a day (Barker & Clevers, 2007). The appearance of an abundance of dividing cells in cancers (Norton, 2007-8; Tomasetti & Levy, 2010) may also be exaggerated as a consequence of stem cell recruitment (Zon, 2008).

Attributing cancers to anything resembling ESCs is all the more difficult, since normally, there are no ESCs in adults. ESCs that appear briefly in the mammalian blastocyst's inner cell mass and embryonic plate exist afterwards only in tissue culture or briefly following re-introduction into blastocysts.

Normally, in amniotic vertebrates, and conspicuously in placental mammals, the first wave of embryonic cells is diverted from embryogenesis toward establishing maternal contact. As the blastocyt implants in the uterus, massive numbers of small cells become motile. Strictly and irretrievably determined, these cells migrate beneath the chorion, fill out chorionic villi, and form the rudiments of a maternal/embryonic exchange system. Gradually, other embryonic plate cells, no longer ESCs, accumulate and fall under local and global commands directing them into germ layers, endo-, ecto-, and mesoderm. Subsequently, endoderm folds into the foregut; endo-mesoderm vesicles converge into the heart-forming region; dermo-myotomes and the neural crest de-epithelialize, and motile cells are released; gonocytes occupy the germinal ridges, and hemocytoblasts colonize liver and bone marrow (see Shostak, 1991). The local and global forces controlling these activities are so powerful that they can even bring small numbers of cancer cells, such as those of teratocarcinomas, into line and direct them toward normal pathways of differentiation in all germ layers (Minsk & Illmensi, 1976).

The possibility that latent ESCs continue in adults seems remote in light of their virtual absence in germ layers and differentiating tissues, but ESCs are sometimes thought to be represented by OSCs, HSCs, and GSCs, and these latent ESCs are even said to be the sources of malESCs. Thus, the notorious EMT is thought to be reminiscent of de-epithelialization in embryonic tissue releasing motile invasive cells. This possibility would seem especially apt for melanomas, malignant schwannomas (neurolemmacytomas) and malignant peripheral nerve sheath tumors (neurofibrosarccomas or triton tumors), all bearing putative ESC markers while resembling retarded embryonic cells differentiating along neural crest lines.

Other malignant cells said to be malESCs are small cells (typically smaller than a red blood cell but larger than a platelet; see Konala et al., 2010), as well as very small embryonic-like stem cells (VSEL-SCs) or small embryonic-like stem cells (SELSCs) expressing "early stem cell markers" such as CXCR4 and CD4, and "signature ESC genes" such as NANOG, a member of the HEDGEHOG-GLI signaling cascade, CD133 (Zbinden et al., 2010), Oct-4, and SSEA-4 (see Zuba-Surma et al., 2010; Sharma & Krishan, 2010). Like embryonic cells, the small malignant cells are multi-potent, differentiating into a variety of tumors from "pediatric sarcomas (e.g., rhabdomyosarcoma, neuroblastoma, Ewing-sarcoma Wilm's tumor) … [to adult] malignancies (e.g., stomach cancer)" (see Kucia et al., 2007). The most aggressive of these are probably small cell lung carcinoma (SCLC aka oat-cell carcinoma) and small-cell carcinomas appearing, if rarely, in the prostate and cervix (Mooi, 2001).

A difference in the number of mutations in two cancers (i.e., in their "mutational burden") provides the best evidence, if only suggestive, for a unique type of cancer cell, albeit not necessarily a malESC. The mutational burden for small-cell lung cancer (Pleasance et al., 2010) is only about half that of a non-small cell lung cancer (Lee et al., 2010). The difference does not seem to be due to mechanisms of mutation or efforts the cells make to correct errors in their DNA, since the frequencies of predominant changes in DNA, such as transversion of $G\bullet C \to T\bullet A$, are similar in both tumors, as are genomic rearrangements and gene translocations. Furthermore, mutation rates in the transcribed strands of DNA are lower than in the non-transcribed strands in both cancers. The different mutational burdens, therefore, would seem due to the small cell lung cancer's cells having accumulated mutations over a shorter period of time than the non-small cell lung cancer's cells. Conceivably, ES-like small cells residing in a dormant state would not accumulate as many mutations as non-small adult cells dividing regularly.

6. Conclusions

The present search for the ancestral branch and root of cancers' stem cells began by testing the merits of opposing hypotheses: A rudimentary stem cell is the ancestor of cancers' stem cells; the stem cells of different cancers evolved in different ancestral cell populations. The first hypothesis proposes that "self-renewal" unifies stem cells, while the second hypothesis proposes that cancers' different stem cells are unrelated.

Unexpectedly, the first hypothesis founders on irreconcilable differences among stem cells. Above all, OSCs and CSCs turn out to be label-retaining cells (LRCs), while HSCs and malHSCs are not (or not demonstrably). Thus, OSCs and CSCs preserve "immortal strands" of DNA and/or divide sluggishly, while HSCs and malHSCs do not preserve "immortal strands" and/or divide comparatively rapidly. What is more, while asymmetrical division occurs in both normal stem cells supporting steady-state populations and reserve cells supporting static cell populations, CSCs and malHSCs have added symmetric division to their modes of cell division (or have fallen back on embryonic habits) while exhibiting the malignant phenotype. In addition, HSCs and malHSCs are vastly more plastic than OSCs and CSCs, and the fate of clones and the disposal of products of terminal differentiation are also different. Thus, "stemness" is different in OSCs and CSCs, on one hand, and HSCs and malHSCs on the other, and stem cells cannot be brought under the umbrella of a unifying concept. The notion of a rudimentary stem cell giving rise to all stem cells must, therefore, be abandoned as without foundation.

Which leaves the possibility that the stem cells of different cancers arose through competition in cell populations. The similarities of CSCs to OSCs in their stem, steady state, attached cubbyhole, and of malHSCs to HSCs in their stem, steady state, unattached cubbyhole fit expectations, but the presence of malignancies in six of the eight categories of cell populations, including non-stem cells (Table 1), suggests that cancer/normal competition went well beyond stem populations. In each of these six categories, the cancer and normal cells have more in common with each other than they have with cells in other categories suggesting that each of these cancer and normal cell pairs arose in a common cell-population ancestor and adopted their normal and malignant phenotypes by competition.

Genomic evidence suggests, moreover, that the evolution of cancers in cell populations is ongoing (Notta et al., 2011; Anderson et al., 2011). For example, competition seems to have trimmed differences between lymphoblastic leukemia and breast cancer cells. Their "transcriptomes" (all the RNA produced in a cell population) or gene expression profiles (demonstrated through laser capture micro-dissection and DNA microarrays) display "extensive similarities" from initiation through progression (Ma et al., 2003) and from original masses to remote metastases (Weigelt et al., 2003). Furthermore, evolution is at work among genetically distinct lymphoblastic leukemia cells. These branch out into multi-clonal cancers, and, in lymphoblastic leukemia, the competitive regenerative capacity of cells growing in immuno-compromised mice (and the prognosis for patients from whom the cells were derived) changes with the tumors' genetic profile.

Of course, the old-fashioned Darwinian methodology employed here cannot say definitively if competition within cell populations gave rise to normal and malignant stem cells, but the evolutionary scenario sketched out here provides a model for future testing. According to this scenario, the evolution of animal cell populations began in symbiotes of epithelial and amoeboid cells in the pre-Phanerozoic. Initially, cell growth was indeterminate, subject only to the availability of resources. Excess cells were simply relegated to propagules of asexual reproduction. But restraints on cell division evolved in response to limitations imposed by animal size. Cellular quiescence or dormancy evolved in animals of small size and brief lifespan and in sequestered tissues, while the steady state evolved in long-lived, large animals and in tissues meeting size constraints while producing new cells in response to stress and contingency. A limiting scaffold determined the number of cells permitted in the steady state population while cell division was permitted to fill gaps.

More subtle controls were required to accommodate turnover in steady state cell populations sustaining cell loss in the process of meeting normal functional demands. Stem cells evolved when niches replaced the scaffold supporting steady state cell populations, and asymmetric division permitted the retention of one out of every two cells produced by division. Epithelial-derived stem cell populations placed a higher priority on controlling cell division than amoeboid-derived stem cells, it would seem, because attached cells are under greater pressure to conform to size limitations than freely moving cells. Thus, steady state CC populations evolved into stem-cell populations when cell division in self-renewing OSCs was constrained by the requirement to divide sluggishly while retaining the "immortal strand" of DNA. Cell division in "primitive" HSCs was not as greatly restrained in evolving self-renewing HSCs presumably due to the ease of disposing of excess cells.

Ultimately, cell populations produced the animals' tissues and organs. CC and OSC populations became organismal surface layers, the substance (parenchyma) of organs,

nerve, smooth muscle, and skeletal muscle equipped with reserve cells. Motile amoeba-like cells became amoeboid archeocytes, interstitial cells, neoblasts, stressed cells, the quiescent cells of connective tissues, and (probably) cardiac myocytes. HSCs' precursors also gave rise to mesenchyme and germ cells, and hemocytoblasts evolved in animals with mesothelial-lined cavities (Hartenstein, 2006). Epithelial and amoeboid characteristics also mixed, for example, as eggs epithelialized by oriented spindles, and amoeboid cells emerged from germ layers by de-epithelialization.

Likewise, cancers evolved through similar competition and selection in six of the eight categories of cell populations. And like their normal counterparts, cancers also mixed epithelial and amoeboid characteristics. For example, metastatic sites collect free cancer-initiating cells (CICs) and produce carcinomas, while the epithelial-to-mesenchymal transition (EMT) creates metastatic, invasive, and destructive amoeba-like cells from carcinomas.

As always, evolution is a push and pull process. Inclusive fitness has deployed successful strategies for neutralizing cancers in most animals. For example, small animals that discharge excess cells in reproductive propagules are not troubled by cancers, and other small animals having turned off growth in adult soma are preadapted to "cancer free" life. This option is not available for large animals, such as human beings, obliged to maintain cell replacement in steady state cell populations at homeostasis, but large animals are not bereft of alternative defenses against cancers. For example, inclusive fitness, it would seem, pushed most human cancers into the time of life beyond the prime reproductive years, and we are also well equipped with massive systemic defenses, such as the immuno-surveillance system. Of course, cancers evolved countermeasures such as recruiting stromal barriers to macrophages, and evolution exapted some cancers with a buffer of slowly dividing stem cells providing stubborn resistance to the best efforts at chemo- and radiotherapy.

Ultimately, competing evolutionary forces may resolve conflict and reach a detente. Thus, some (ancient?) malignant and normal cell populations would seem to have reached equilibrium (e.g., adenomas derived from CCCs) and many cancers are all but unknown except when induced by radiation, carcinogens, etc. On the other hand, highly malignant cancers (e.g., melanomas and small cell lung cancers) are far from equilibrium and may be newly evolving or easily provoked by conditions of contemporary life.

In sum, Dobzhansky has been vindicated, and the light of evolution brightens the outlook for making sense of cancer. Most importantly, researchers equipped with an evolutionary perspective may now be able to devise effective strategies for preventing cancers, detecting them early, and bringing those cancers that cannot be prevented into equilibrium with normal tissues.

Obviously, cancer's should not be given a competitive edge through exposure to anthropogenic carcinogens such as those in cigarette smoke, air pollutants, the polynuclear aromatic hydrocarbons, chlorinated hydrocarbons, pesticides, and/or metals in effluvia and food. Researchers should seek clues for prevention in the prophylactic devices deployed against cancers by most animals and our own young. Researchers hoping to detect, monitor, and track cancers should also take a hard look at perturbations in the biometrics of normal tissues competing with neoplasm rather than relying solely on the detection of cancer's markers. Finally, by correcting cancers' equation of state for competition and selection, cancers' evolution in the past should be plotted and steps taken to thwart the flow of cancers' evolution in the future.

7. References

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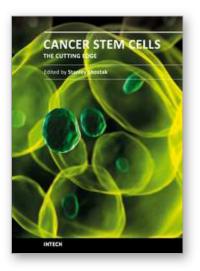
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Cancer Stem Cells - The Cutting Edge

Edited by Prof. Stanley Shostak

Over the last thirty years, the foremost inspiration for research on metastasis, cancer recurrence, and increased resistance to chemo- and radiotherapy has been the notion of cancer stem cells.The twenty-eight chapters assembled in Cancer Stem Cells - The Cutting Edge summarize the work of cancer researchers and oncologists at leading universities and hospitals around the world on every aspect of cancer stem cells, from theory and models to specific applications (glioma), from laboratory research on signal pathways to clinical trials of bio-therapies using a host of devices, from solutions to laboratory problems to speculation on cancers' stem cells' evolution. Cancer stem cells may or may not be a subset of slowly dividing cancer cells that both disseminate cancers and defy oncotoxic drugs and radiation directed at rapidly dividing bulk cancer cells, but research on cancer stem cells has paid dividends for cancer prevention, detection, targeted treatment, and improved prognosis.

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