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CANCER STEM CELLS: A NEW PARADIGM FOR DEVELOPING SELECTIVE BRAIN TUMOR TREATMENTS

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Central Nervous System (CNS) tumors are one of the most prevalent forms of cancer in the U.S. and are the leading cause of cancer deaths in children (1-3). Current therapies can affect all cells, as with radiation, or highly proliferative cells, as with many chemotherapy regimens. While these treatments are often effective in shrinking the tumor mass, they can cause unintended toxicity to normal tissues. Furthermore, the tumors can recur, suggesting that some tumor cells have ways of escaping the treatments. Recent studies indicate that cancers contain a subpopulation of stem-like cells that maintain tumor growth, and that may elude the treatments currently used.

For decades, tumor biologists had noted the similarities between cancer cells and the primitive cells that populate the developing fetus (4). One school of thought proposed that, as mature cells accumulate enough mutations, they regressed to a primitive state that resembled early development and led to cancer. However, since the majority of cells in many organs (skin and blood, for example) live only for a short period of time before being replaced, other biologists suggested that cancer would have to arise from cells that were already primitive and residing in the adult organ long enough to accumulate the necessary mutations.

In fact, the discovery of such primitive cells came from studies done in the wake of the atomic bombing of Hiroshima and Nagasaki, where many survivors of the bombing eventually succumbed to radiation sickness. Subsequent experiments showed that radiation damage to the immune systems of mice and humans were reversed by grafting bone marrow from a donor (5). In the early 1960s, studies by James Till, Ernest McCulloch and colleagues (6) led to the positive identification of the reconstituting cell type. Termed a stem cell, it was defined by the capability to self-renew throughout life, generate multiple daughter cell types, and regenerate the organ system in which it resides. In the decades after, other organs such as the brain were found to maintain their own specific stem cells (7). This finding was particularly significant since no new nerve cells were thought to be born in the brain after development was completed. There is evidence that postnatal (after birth) brain stem cells are actually the remnants of the stem cell population that initially generates the brain (8, 9).

Postnatal stem cells have many unusual features. First, they divide only infrequently (termed quiescence); the majority of cell division occurs by the immediate daughters of stem cells, called transit amplifying cells (10-12). But all progeny are part of a clone, i.e. the descendants of that original stem cell. Second, they reside in a very specialized region, called a niche, which consists of more mature cells that control the stem cell division rate and the types of daughter cells generated (13). Third, stem cells have surface proteins (called ABC transporters) that pump out a wide variety of chemicals that would otherwise cause the stem cell to differentiate or die (14). These unique features have relevance to the properties of cancer.

Over the last decade, researchers have studied cancer by exploiting the fact that stem cells have different molecules (termed markers) on their surface than do other cells. This property allows them to be physically separated and purified from other cells in a process called cell sorting (15). John Dick and colleagues found that a very small fraction of leukemia cells had a combination of surface markers that appeared quite similar to stem cells. They sorted and injected these cells into mice, then measured tumor growth compared with mice injected with tumor cells that did not have this combination of markers (16). They found that tumors were only regenerated from the cells that expressed the stem cell markers. Furthermore, the resulting leukemia contained all the heterogeneous cell types typical of that disease, despite the homogeneity of the starting cells. Finally, a small subpopulation of the leukemia cells retained stem cell markers and could be re-sorted and re-injected to generate a new tumor. Thus, these rare cells were reconstituting, multipotent and self-

renewing, thereby fulfilling the definition of stem cells. Subsequently, these methods were used to identify “cancer stem cells” in other organs (17), including the brain tumors glioblastoma, medulloblastoma and ependymoma (18, 19). While it is difficult to disprove the possibility that more mature cells can regress and re-express these markers, these studies strongly support the idea that cancer is caused and maintained by stem cells that become dysfunctional.

These findings have generated much excitement because they explain aspects of tumor growth that confound current therapies. First, most anti-cancer treatments target DNA replication (20) or signals that initiate cell division (21). They therefore preferentially affect the most rapidly proliferating cells in the body rather than the relatively quiescent stem cells (10-12). Second, tumors are heterogeneous but are thought to form by clonal growth from a single starting cell, just as diverse cell types are normally generated from stem cells. Third, cancer chemotherapy is often impeded by multidrug resistance, mediated by ABC transporters (22, 23) that are often expressed at highest levels in stem cells (24). Thus, stem cells appear to have characteristics that render them resistant to anti-proliferative chemotherapies. This is particularly important for infant CNS tumors, where surgery is not advised because of unacceptable neurocognitive results and the currently used anti-cancer drugs yield a 34% 3-year survival rate for pediatric neuroectodermal tumors (PNETs) (3).

Translation of these findings to new cancer treatments depends on identifying exploitable differences between cancer stem cells and their normal counterparts. Some progress has been made in non-brain cancers. Acute myeloid leukemia-initiating cells from patients were found to differ from normal stem cells by the lack of surface c-Kit expression (25). The loss of the tumor suppressor gene *Pten* turned normal mouse immune system stem cells into leukemia stem cells, but these were more sensitive than normal stem cells to induced death by the drug rapamycin (26). Genomic (gene expression) and proteomic (protein expression) comparative studies are currently underway to identify candidate targets for brain tumors. Tumor reconstitution assays in mice and cell culture clonal assays allow normal and cancer stem cells to be compared for selective vulnerabilities.

The stem cell niche is also a potential target for cancer therapies (27, 28). Medulloblastoma and ependymoma stem cells selectively associate with endothelial cells (29), a component of blood vessels that are known to promote normal stem cell function (13, 30-32). Drugs that inhibit the growth of these blood vessels can prevent tumor growth when the tumor cells are artificially induced to recruit blood vessels (29). This supports other findings that tumors may become addicted to certain microenvironments by their mutations, which sensitizes them in ways that normal stem cells are not (33). Future therapies may involve cocktails that simultaneously target both the cancer stem cells and their niche.

These results suggest that drugs can be developed, or may already exist, that can kill cancer stem cells without harming normal stem cells. Since the tumor is dependent on cancer stem cells for replenishment of the cancer population, treatments that selectively kill cancer stem cells will deprive the tumor of its regenerative capacity. The remaining non-stem tumor mass may be removed by surgery or simply left to regress on its own, and non-cancer cells will be spared by the more selective treatment. While this area of research is relatively new, it ties together many previously disparate observations about cancer cells and provides a framework for developing more selective and effective treatments for brain tumors.

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