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EDITORIAL

Dueling models in head and neck tumor formation

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The two leading models that have been used to explain tumor progression in head and neck squamous cell carcinoma (HNSCC) are the stochastic clonal evolution model, in which many tumor cells are individually capable of recapitulating the entire tumor mass, and the cancer stem hierarchy model, in which only rare totipotential tumor stem cells can recapitulate the tumor. In this issue, Cameron *et al* use cell surface marker and clonal cell analyses in combination with a xenotransplant approach to provide data that support the stochastic clonal evolution model in HNSCC. This interpretation is subject, however, to limitations inherent in the experimental approach employed. Understanding the basis of tumor progression in HNSCC as well as other cancers should be further explored because of important implications for effective treatments.
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Similar to other cancers, head and neck squamous cell carcinoma (HNSCC) is characterized by the phenotypic heterogeneity among the cellular constituents.[1,2] The two leading models of tumor progression in HNSCC are the 'stochastic clonal evolution' model and the 'cancer stem cell (CSC) hierarchy' model. In this issue, Cameron *et al*[3] directly address this controversial topic. Using a xenotransplant approach to laboratory mice, Cameron *et al*[3] show that single cells derived from HNSCC cell lines consistently have tumor-initiating activity. In addition, clonal variants derived from tumor cells give rise to microenvironments that support tumor cells. These observations are most consistent with the stochastic clonal evolution model.

TWO MODELS OF TUMOR PROGRESSION IN SOLID TUMORS

The CSC theory was proposed nearly a half century ago, soon after the discovery of hematopoietic stem cells. According to the CSC model, cancer can be understood by application of the principles of stem cell biology. Stem cells have been identified in most tissues, including bone marrow, brain, intestine, and skin, in which tissue structure is generated by hierarchical cell systems. A similar hierarchical organization, with a CSC at the apex, exists in cancer tissues. Small numbers of CSCs maintain the tumor through proliferation and generation of more differentiated cells that go on to form the tumor mass. Support for the CSC theory came from early studies (in 1963), in which it was reported that only 1–4% of murine lymphoma cells have the capacity to form colonies in spleen; similarly only 0.02–0.1% of solid tumor cells are able to form colonies.[4] Rare human leukemic cells can generate acute myeloid leukemia in NOD/Scid mice,[5] and marker analysis reveals that leukemogenic capacity is found in CD34-positive and CD38-negative cell fractions. These reports suggest that, similar to intact hematopoiesis, leukemic cells exhibit a hierarchical system of the potential to form tumors, with CSCs at the top.

In contrast, the stochastic clonal evolution model posits that all tumor cells have equal ability to propagate the tumor. Most tumors are composed largely of cells with some degree of differentiation. This morphological heterogeneity is explained by aberrant differentiation pathways resulting from genetic and/or epigenetic instability of the tumor cells. Despite this heterogeneity, according to the stochastic clonal evolution model all cells are capable of giving rise to subsequent tumors.

THE CLONAL EVOLUTION MODEL REVISITED IN HNSCC

A CSC is defined as a cell within a tumor that possesses the capacity to both self-renew and to

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yield the various heterogeneous lineages of cancer cells that comprise the tumor. CSCs can thus only be defined experimentally by their ability to recapitulate the generation of a continuously growing tumor.[6] In this study, Cameron *et al*[3] used xenotransplantation, cell surface marker expression, and clonal cell analysis to examine the biologic basis for tumor progression and intratumoral heterogeneity in HNSCC.
The self-renewal capability of CSCs can be assessed by a colony-forming assay and tumor formation in irradiated and/or immunodeficient mice. Implantation into immunodeficient mice is more reliable, but certain biological factors render the interpretation of the xenotransplantation assay difficult. Results are influenced by the success of tumor cell homing and engraftment. Poor tumor initiation may result from post-transplant loss of the implanted cells. In the Cameron paper, GFP labeling to trace implanted cells obviated this possibility.[3]

Isolated subpopulations of tumor cells with stem cell-like features can form solid tumors *in vivo*. To identify solid tumor CSCs, tumor cells are fractionated using cell surface markers and implanted into immunodeficient mice, after which xenograft growth and cellular composition are assessed. Cell surface molecules, such as CD24, CD44, and CD133, are often used to identify cell populations containing CSCs. In breast cancer, $CD24^{low/negative}$ $CD44^{positive}$ cell populations have high tumorigenic potential in immunodeficient mice.[7,8] In brain tumors, CSCs are identified combining the use of the CD133 marker and the 'side population,' defined as cells that actively exclude dyes such as Hoechst 33342.[9] Similar findings have been reported in a wide variety of tumors originating from prostate, colon, pancreas, liver, and melanocytes.[10–13] In addition to cell surface markers, aldehyde dehydrogenase 1A1 and cystatin E/M are suggested as CSC markers of the prostate and brain, respectively.[14,15] Controversial results are reported in brain tumors, in which both CD133-positive and CD133-negative populations have CSC properties.[16] In HNSCC, a CD44-positive population is reported to possess CSC properties.[17] By contrast, in this issue, Cameron *et al*[3] report no correlation in HNSCC between the expression of specific markers (CD44, CD133, side population) and cells with tumor-initiating activities, a result that favors the clonal evolution model.

The variability in results may be due in part to limitations inherent to the experimental approaches used. Isolation of CSCs, especially from solid tumors, is relatively difficult, and can be carried out successfully only by flow cytometric sorting using antibodies. Instead of tumor tissues, Cameron *et al*[3] used cell lines and subcloned cells from single cells. Single cell clones randomly isolated from HNSCC cell lines were all capable of initiating tumors after implantation into mice (Cameron *et al*).[3] This result provides support for the clonal evolution model. It is to be noted, however, that after subcloning, cells were propagated for two to five passages *in vitro* before the implantation. This raises the concern that propagation may somehow have altered the tumor cells, imbuing them with tumor-initiating activity. Alternatively, tumor cells lacking such activity may not have been successfully cultured. Thus, interpretation of results derived from xenotransplantation experiments that use cultures derived from single cells must be made with caution. Furthermore, tumor initiation in HNSCC xenotransplantation systems is inefficient, requiring 3 to 6 months of monitoring after implantation. Overcoming these methodological difficulties would allow for more robust experimental tests of the two models.

NICHE CELLS DERIVED FROM TUMOR CELLS

Interactions of tumor cells with their microenvironment can lead to altered growth and differentiation. The phenotypic plasticity of tumor cells suggests that dynamic equilibrium exists between CSCs and non-CSCs, which is dependent on signals from the microenvironment.[18] Leukemic stem cells express high CD44 and the CD44-mediated interaction between cancer cells and their microenvironment has led to the development of specific antibody therapy targeted to CD44.[19,20] Anti-CD44 therapy inhibits the homing and engraftment of leukemic stem cells but not of hematopoietic stem cells. Similarly, engraftment of solid tumors may be affected by CD44 expression on supporting cells (microenvironment), and CD44 is also a cell surface marker for CSCs in solid tumors such as breast cancer.

Interactions of stromal cells with tumor cells include effects on growth stimulation, angiogenesis, and immunocompetence. From this point of view, tumor cells imitate normal tissues, in that stromal cells support tissue stem cells. These stromal cells can also be derived from tumor cells. Clonal variant-derived tumor cells can act as stromal supporting cells and can modulate overall tumor initiating activity (Figure 1). Cameron *et al*[3] proposed that the ability to initiate tumors is

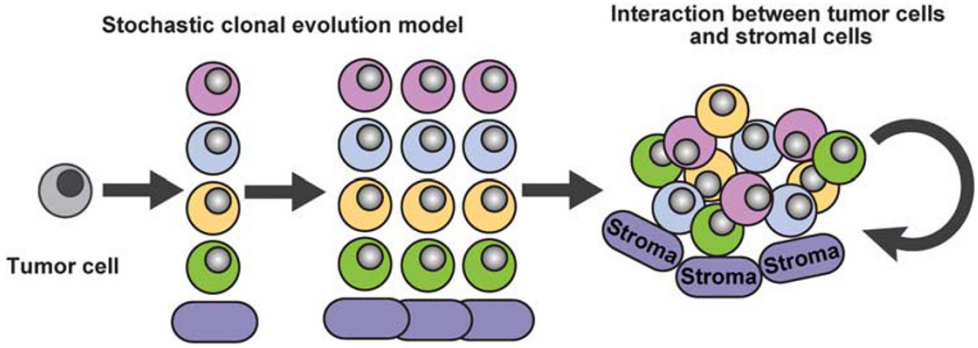

Figure 1 Scheme of the stochastic model. In the model proposed by Cameron *et al*[3], clonal variants, including stromal cells derived from tumor cells, generate a microenvironment for tumor cells, and support tumor progression after tumor cells undergo clonal evolution.

influenced by the ability of tumor cells to render the stromal environment permissive to tumor growth.

Although the experiments by Cameron *et al* were carried out using cell lines and their single cell-derived subclones, the results, which support the clonal evolution model of HNSCC should be carefully considered in light of the therapeutic implications. The two models of tumor formation suggest fundamentally different approaches in the treatment of HNSCC. The CSC hierarchical model suggests that CSCs are the only relevant target for therapy. In contrast, the clonal evolution model suggests that all tumor cells must be targeted, as all are equally able of causing relapse after therapy. Analyses of primary uncultured HNSCC cells using the approaches used in the study by Cameron *et al* will greatly advance our understanding of which tumor formation model is most applicable to HNSCC.

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DISCLOSURE/CONFLICT OF INTEREST

The authors declared no conflict of interest

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