The Niche Architecture and Structure of Cancer Stem Cells

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Background: Cell nuclei with Feulgen-negative round areas of uniform diameter in the buccal mucosa were first reported by the author in 1962 as reflections of a primary malignant tumor in a distant site as well as in precursor lesions of the uterine cervix. These cells were designated as a malignancy-associated change (MAC). The origin of these cells was unknown. However, recent observations reveal that the nuclear structure of cancer stem cells is identical to MAC cell morphology. The nuclear pattern originally identified as MAC denotes a cancer stem cell at a distance from the primary tumor. **Methods:** This coincidental finding led to the review of 76 histologic sections of adenomatous colonic polyps, 60 specimens of peripheral blood and 299 buccal mucosal spreads. Evaluation of the cell nuclear structure was made by utilization of the highest microscopic magnification (100x objective, 10x ocular, tube length 1.25). **Results:** Review of the MAC cases revealed the cancer stem cell microenvironment with asymmetric cell division. The stem cell niche has a well organized but variable microenvironmental architecture depending upon developmental stages within the niche. Self-renewal daughter and progenitor cells were characterized by identical cribriform nuclear structure in Feulgen-negative stained and hematoxylin-eosin unstained spherical areas with uniform diameters. These malignancy-associated stem/progenitor cells were identified in 19 of 28 cases of colonic adenomatous polyps with focal carcinoma at sensitivity of 68%, specificity 88%. Peripheral blood cells with identical nuclear pattern were observed as a manifestation of carcinomas of the bladder, kidney and prostate in 23 of29 cases at sensitivity 79%, specificity 80%. In buccal mucosa specimens an identical nuclear pattern was noted in112 of 145 cases at sensitivity 77%, specificity 86% as a reflection of tumors in the lung, urogenital and gastrointestinal tract. **Discussion:** The specific uniform nuclear structure in cells of the colon, peripheral blood and buccal mucosa distant from the primary tumor were identified as cancer stem cells. Self-renewal and progenitor cells in asymmetric division have an identical specific nuclear structure and retain their precise nuclear pattern during their migration and final destination at distant sites. The nuclear-specific structure of these cells permits not only their identification but also their role as a reflection of a primary tumor at a different site. **Conclusion:** Examination at the highest magnification of the light microscope revealed precise nuclear structure of self-renewal and progenitor cells. Visualization of the cancer stem cell niche architecture and of the stem cell specific nuclear structure offers additional parameters for identification of stem/progenitor cell morphology, migration pathways and detection of cancer manifestation in sites distant from the primary tumor.

Background

Past observations of cellular changes in cancer patients identified cells with numerous round, Feulgen-negative and hematoxylin-eosin (H&E) unstained areas. The origin of these cells was unknown but their significant correlation with a primary tumor at a distant site was interpreted as a malignancy-associated change (MAC) [1] . Current observations of cancer stem cells revealed that Feulgen-negative areas consisted of numerous spheres in contrast to patients without cancer who had cells devoid of spheres.

The residing location of cancer stem cells is the stem cell microenvironment — the niche. In the niche the cancer stem cell is surrounded by a moderate to large halo formation with an inner membrane (β-catenin) and outer membrane (N-cadherin). The supporting tumor or mesenchymal cells are molded tightly to the adjacent halo.

Method

A high magnification of the light microscope (x100 objective, x10 ocular) was utilized for identification and interpretation of cellular structures. The Feulgen-reaction and hematoxylin-eosin (H&E) stain were applied for buccal mucosa spreads, the H&E stain for histologic sections and the Wright-Giemsa stain for blood smears.

The study included 28 cases of colon adenocarcinoma, and routinely collected specimens (29 peripheral blood smears and 145 buccal mucosal spreads). The control specimens consisted of epithelial cells that had been identified in histologic sections at the same magnification with an additional 20-fold enlargement in correlation with the histologic pattern (Fig. 9).

Acknowledgements

William Munger, Ph.D. for his generous assistance and advice in the preparation of this poster presentation.

I. STEM CELL NICHE

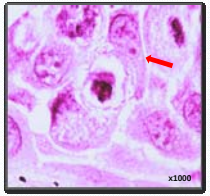

Fig 1: Stem cell niche: arrow identifies support cell. (Mild intraepithelial neoplasia of uterine cervix)

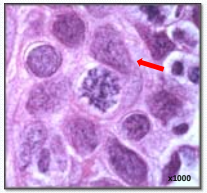

Fig 2: Stem cell niche with progenitor cell: arrow identifies support cell. (Infiltrating carcinoma of uterine cervix)

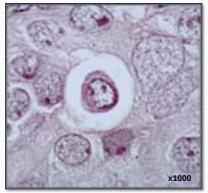

Fig 3: Stem cell niche: Halo formation between inner and outer membrane. (Medullary carcinoma of breast)

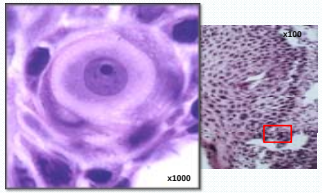

Fig 4: Stem cell niche: Cell in quiescent state; halo formation between inner and outer membrane; niche surrounded by closely attached support cells. (High-grade intraepithelial neoplasia)

II. SELF-RENEWAL DAUGHTER CELLS

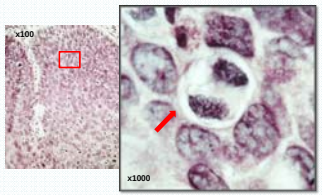

Fig 5: Niche with assymetric division: Niche surrounded by support cells with one self-renewal daughter cell (arrow) consisting of spheres and one differentiated tumor cell. (Infiltrating squamous cell carcinoma of uterine cervix [2])

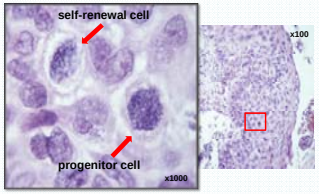

Fig 6: Assymetric division: Self-renewal daughter cell and progenitor cell consisting entirely of spheres with uniform luminal diameters. (Papillary adenocarcinoma of uterine cervix [3])

III. CELL MIGRATION

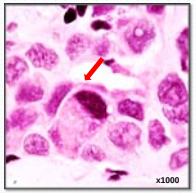

Fig 7. Niche with single self-renewal daughter cell (arrow). (Papillary carcinoma of urinary bladder)

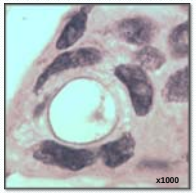

Fig 8: Empty niche surrounded by support cells.

IV. NO SPHERES

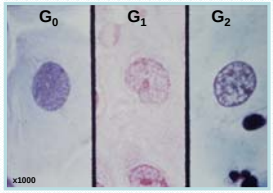

Fig 9: Normal epithelial cells at cell cycle checkpoints.

V. SPHERES IN MIGRATING CANCER STEM CELLS

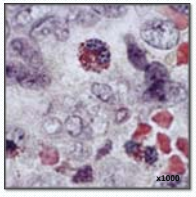

Fig 10: Migrating stem cell with rugged border. Cell consists of uniform-sized spheres. (Adenocarcinoma colon)

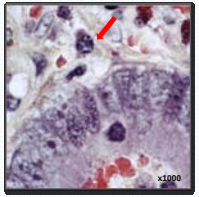

Fig 11: Migrating stem cell (arrow): Cell consists of uniform-sized spheres. (Adenocarcinoma colon)

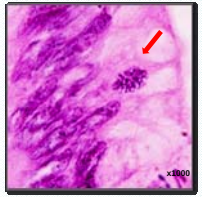

Fig 12: Migrating stem cell (arrow): Cell consists of uniform-sized spheres. (Benign area adjacent adenocarcinoma colon)

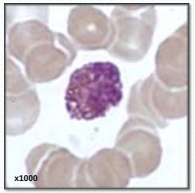

Fig 13: Migrating stem cell with rugged border. (Blood smear from patient with breast cancer)

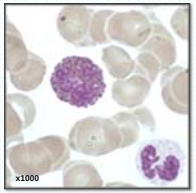

Fig 14: Migrating stem cell with rugged border. (Blood smear from patient with uterine cervix cancer)

VI. SPHERES OF MALIGNANCY-ASSOCIATED CHANGES IN BUCCAL CELLS

Fig 15: Prominent spheres with uniform diameters. (Patient with lung cancer)

Fig 16: Prominent spheres with uniform diameters. (Patient with adenocarcinoma colon)

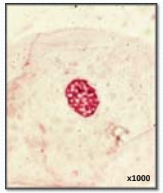

Fig 17: Prominent spheres with uniform diameters. (Patient with ovarian cancer)

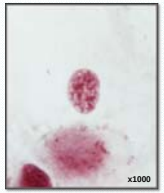

Fig 18: Prominent spheres with uniform diameters. (Patient with recurrent mucus cyst adenocarcinoma)

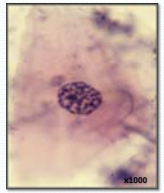

Fig 19: Prominent spheres with uniform diameters. (Patient with gastric adenocarcinoma)

VII: CULTURE

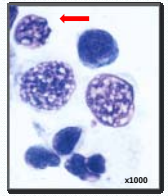

Fig 20: Prominent spheres with uniform diameters similar to buccal cells (arrow). (Circulating leukemic DNA-induced spheres)

Results

The cancer stem cell niche may contain cells with nuclei either in quiescent state or in symmetric or asymmetric division. Asymmetric cell division of the cancer stem cells — within the remaining unstained area of the halo — displayed the self-renewal daughter cell consisting entirely of spheres with uniform luminal diameters, a differentiated tumor cell or a progenitor cell containing spheres with a slightly smaller diameter than the self-renewal cell. In adenocarcinoma of the colon, cancer stem cells were identified in 19 of 28 cases with a sensitivity of 85% and specificity of 88%. In buccal mucosa spreads, cancer stem cells were found in 112 of 145 cases with sensitivity of 77% and specificity of 86%. The cancer stem cells may leave the niche and migrate to distant sites. In their migration through tumor tissue and blood, the progenitor or self-renewal cells maintain their structure of spheres with uniform diameters throughout migration. Buccal mucosal cells regardless of the type/site of an associated tumor have identical spheres with uniform diameters.

Discussion

Spheres with large diameters may develop, for example, as a result of circulating leukemic DNA (Fig. 20. Anker P, Stroun M, Nieburgs H, unpublished observation) in contrast to spheres with considerably smaller diameters in normal phase G_0 of the post mitotic cell-cycle checkpoint. In the cancer stem cell niche the well-defined physical structure around the nucleus may present the feature that protects the stem cells from adverse extraneous influences and may explain the resistance of the niche cells to conventional therapeutic agents. In the current review spheres in differentiated buccal mucosa cells are identical to Feulgen-negative areas that were observed in MAC. The finding of cancer stem cells in buccal mucosa offers a morphological marker for cancer detection of an overt or occult malignant neoplasm at a distant site.

References

[1]Nieburgs HE, Herman BE, Reisman H. Buccal cell changes in patients with malignant tumors. Lab Invest 1962;11(1):80-88.
[2]Nieburgs HE. Tissue and cell pathology of uterine cervix dysplasias and carcinoma in situ. Acta Cytol 1971;15(6):513-532.
[3]Nieburgs HE. Recent progress in the interpretation of Malignancy-Associated Changes (MAC). Acta Cytol 1968; 12(6):445-453.