Molecular & Cellular Oncology 2:1, e969634; January/February/March 2015; Published with license by Taylor & Francis Group, LLC

The microenvironment reprograms circuits in tumor cells

Qingchun Cai and Yan Xu*

Department of Ob/GYN; Indiana University School of Medicine; Indianapolis, IN USA

Keywords: anoikis resistance, cell survival, floating cells, glycolysis, microenvironment, ovarian cancer, SRC

In the course of multistep oncogenesis, initially normal cells acquire several new functions that render them malignant. We have recently demonstrated that the peritoneal microenvironment promotes resistance to anoikis in ovarian cancer cells by reprogramming SRC/AKT/ERK signaling and metabolism. These findings have prognostic and therapeutic implications.

In 2000, Hanahan and Weinberg initially proposed 6 cancer hallmarks: sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis.[1] The subsequent remarkable progress in cancer research, with more than 1.5 million cancer-related papers published since the year 2000, promoted their proposal of the next generation of hallmarks of cancer in 2011.[2] Four emerging hallmarks, including deregulating cellular energetics, have been elucidated. Together with an emphasis on intracellular signaling networks within several functional circuits (proliferation, motility, viability, cytostasis, and differentiation) and a commentary section on the tumor microenvironment, our understanding of the cancer orchestra has been described at a new level.

While most tumor cells acquire several hallmarks of cancer during multistep development, not all hallmarks and cellular signaling/functional circuits are likely to be involved at every step of cancer development. In fact, one or a few of these hallmarks or circuits are likely to be differentially regulated and switched on and off at different steps of cancer development to suit the stage-specific pathologic requirements.

To determine the critical processes of ovarian cancer (OC) development, we have developed a highly aggressive OC cell line (ID8-P1) from a less aggressive mouse OC cell line (ID8-P0) through *in vivo* passage in C57BL6 mice.[3] *In vivo* tumor characterization and *in vitro* mechanistic assays reveal that enhanced anoikis resistance (but not increased proliferation, migration, or invasion) is a critical process responsible for this aggressiveness.[3]

Our data have important implications on several levels. First, the changes associated with enhanced aggressiveness are induced by the tumor microenvironment, since *in vitro* cell culture did not induce these alterations. In addition to various host/stromal/immune cells, acellular components in the microenvironment also play critical roles in regulating cancer cells. These include, but are not limited to, insoluble extracellular components, such as extracellular matrix proteins, and soluble components, such as acellular vesicles (i.e., exosomes), secreted proteins, peptides, bioactive lipids, and other factors. In addition, gaseous components, including O_2 and NO, play important roles in regulating the properties of cancer cells. Both genetic and epigenetic changes in ID8 cells may occur, which appear to be stable; after at least 20 *in vitro* passages, the aggressive tumorigenic potential of ID8-P1 cells is stable. Our results also emphasize the potential differences in cellular properties in 2-dimensional cell culture systems *versus* the *in vivo* environment.

Second, our data indicate that stresses, including hypoxic conditions and growth as a cell suspension in the peritoneal cavity, have specifically reprogrammed the cellular signaling circuits by enhancing the viability circuit and by maintaining or even suppressing other circuits, such as proliferation and motility. This is evidenced by the increase in anoikis resistance, but not in the proliferative, migratory, and invasive capacities *in vitro* and *in vivo*; by the tight correlation between the number of surviving peritoneal floating cells and the overall aggressiveness of tumor development; and by signaling pathway analyses.[3] Our data suggest that when cells encounter great stresses in the microenvironment, they switch off other cellular activities to ensure their survival. Even though cell migration and invasion into surrounding tissues and the subsequent colonial

*Correspondence to: Yan Xu; Email: xu2@iupui.edu
Submitted: 08/27/2014; Revised: 08/28/2014; Accepted: 08/28/2014
http://dx.doi.org/10.4161/23723548.2014.969634

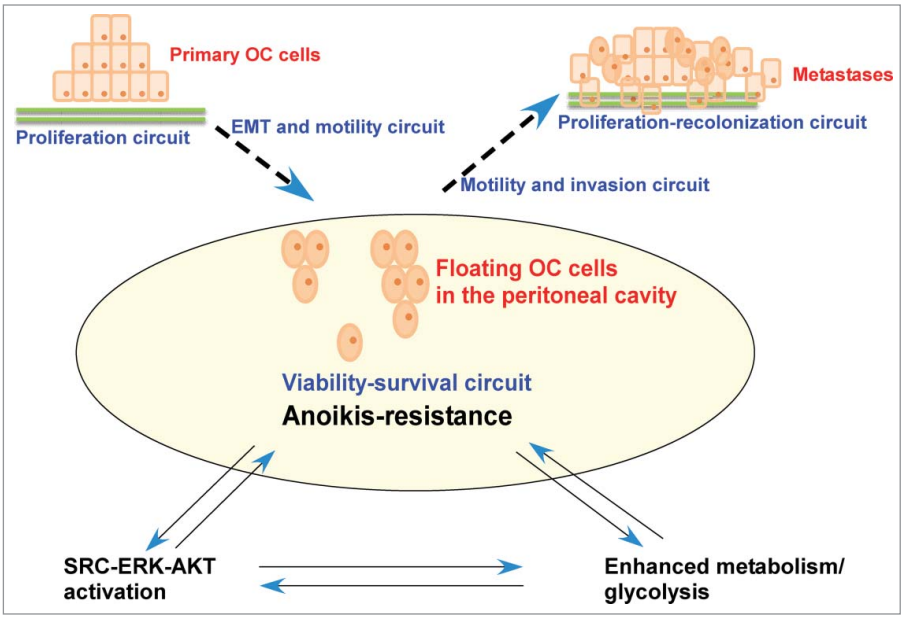

Figure 1. Signaling switches during ovarian carcinogenesis. Primary ovarian cancer (OC) cells, derived from either ovarian surface epithelial cells or fallopian tube epithelial cells, primarily acquire a proliferation circuit to develop into primary tumors. Reduced cell-to-cell contact and possible induction of the epithelial–mesenchymal transition (EMT), together with enhanced cell motility, are probably involved in the dissemination of OC cells from the primary site to the peritoneal cavity, where the tumor cells encounter a hostile microenvironment. The lack of a blood supply and supporting extracellular matrix leads to decreased oxygen concentrations (hypoxia) and nutritional supplies, as well as the need to grow as a cell suspension. These conditions reprogram the cells into a survival circuit, in which the SRC-AKT pathway and increased glycolysis play crucial roles. The number of surviving peritoneal floating cells directly correlates with how quickly and to what extent metastases will develop, and thus these cells may represent the best cancer development markers and therapeutic targets. Once tumor cells attach to the secondary sites, another circuit switch may occur in order for them to generate sizable metastases.

growth at these sites are the primary causes of morbidity and mortality of most cancer patients,[4] the number of cells surviving against initial stresses in the peritoneal cavity is the key determinant of how quickly and to what extent the tumor will develop.[3]

Third, we have identified that the SRC/ERK/AKT/ signaling pathway plays a pivotal role in anoikis resistance. This property is not only necessary, but also sufficient to promote cell survival in suspension in the peritoneal cavity,[3] which is particularly meaningful for OC, where a large amount of living tumor cells are present in patients' ascites. For many other solid tumors, metastasis is mainly mediated via the blood stream. Similar viability circuit switches may exist in such cases, although the signaling molecules may vary because the stresses in the blood stream are likely to be different from those in the peritoneal cavity.

Another important feature of the aggressive ID8-P1 cells is the increase in metabolism, and in glycolysis in particular.[3] Although Otto Warburg first proposed the concept of an aerobic glycolysis switch in cancer cells as early as the 1920s, the importance of this concept in terms of understanding cancer origin and signaling mechanisms, in addition to designing better targeting strategies, has only emerged in recent decades.[2] The genomic instability observed in tumor cells and all other recognized hallmarks of cancer have been considered downstream epiphenomena of the initial disturbance of cellular energy metabolism.[5] We have also revealed the connection between the SRC pathway and glycolysis, both of which are involved in the viability circuit.[2,3] SRC may also affect glycolysis by targeting other mitochondrial components.[6] Although the only "metabolic" drug in clinical use is still the enzyme L-asparaginase, the increasing amount of experimental evidence, combined with the number of trials in progress, suggests that metabolic drugs will soon complement standard anticancer chemotherapy and modern biological drugs.[7,8] **Figure 1** is a schematic and perspective view of our findings.

Finally, our studies have several clinical implications: 1) co-targeting the host environment is important in OC; 2) the peritoneal floating OC cells may have value as markers for diagnosis, prognosis, and/or personalized treatment; 3) removing ascites along with the floating cells could be beneficial for patients with OC; and 4) targeting a specific vulnerable stage of tumor development and a critical circuit may be an effective approach to therapy development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgment

We would like to thank Mr. Kevin J. McClelland for editing the manuscript.

Funding

This work is supported in part by the National Institutes of Health (RO1 155145 to YX) and the Mary Fendrich-Hulman Charitable Trust Fund to YX.

References

1. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100:57-70; PMID:10647931; http://dx.doi.org/10.1016/S0092-8674(00)81683-9
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144:646-74; PMID:21376230; http://dx.doi.org/10.1016/j.cell.2011.02.013
3. Cai Q, Yan L, Xu Y. Anoikis resistance is a critical feature of highly aggressive ovarian cancer cells. Oncogene 2014; PMID: 25132267; http://dx.doi.org/10.1038/onc.2014.264
4. Sporn MB. The war on cancer. Lancet 1996; 347:1377-81; PMID:8637346; http://dx.doi.org/10.1016/S0140-6736(96)91015-6
5. Seyfried TN, Flores RE, Poff AM, D'Agostino DP. Cancer as a metabolic disease: implications for novel therapeutics. Carcinogenesis 2014; 35:515-27; PMID:24343361; http://dx.doi.org/10.1093/carcin/bgt480

6. Hebert-Chatelain E, Jose C, Gutierrez Cortes N, Dupuy JW, Rocher C, Dachary-Prigent J, Letellier T. Preservation of NADH ubiquinone-oxidoreductase activity by Src kinase-mediated phosphorylation of NDUFB10. Biochimica et biophysica acta 2012; 1817:718-25; PMID:22321370; http://dx.doi.org/10.1016/j.bbabio.2012.01.014
7. Chiu M, Ottaviani L, Bianchi MG, Franchi-Gazzola R, Bussolati O. Towards a metabolic therapy of cancer? Acta bio-medica : Atenei Parmensis 2012; 83:168-76; PMID:23762991
8. Suh DH, Kim MK, No JH, Chung HH, Song YS. Metabolic approaches to overcoming chemoresistance in ovarian cancer. Ann New York Acad Sci 2011; 1229:53-60; PMID:21793839; http://dx.doi.org/10.1111/j.1749-6632.2011.06095.x