Cancer Biology & Therapy 11:8, 708-713; April 15, 2011; © 2011 Landes Bioscience

Ovarian cancer stem cells and inflammation

Gil Mor,* Gang Yin, Ilana Chefetz, Yang Yang and Ayesha Alvero

Department of Obstetrics, Gynecology and Reproductive Sciences; Reproductive Immunology Unit; Yale University School of Medicine; New Haven, CT USA

Key words: ovarian cancer stem cells, inflammation, toll-like receptors, nuclear factor kappaB, ovarian cancer, recurrence

Epithelial ovarian cancer (EOC) is the fourth leading cause of cancer-related deaths in women in the United States and the leading cause of gynecologic cancer deaths. The major limiting factor in the treatment of ovarian cancer is recurrence and chemoresistance. Individuals who succumb to advanced-stage ovarian cancer inevitably become refractory to chemotherapy, resulting in disease progression and death. The source of recurrence and lack of response to chemotherapy is unknown. The focus of this review is to evaluate the question of recurrence and chemoresistance based on the concept of the cancer stem cells and inflammation.

Epithelial ovarian cancer (EOC) is the fourth leading cause of cancer-related deaths in women in the United States and the leading cause of gynecologic cancer deaths.[1,2] Approximately 80% of patients with primary disease respond to surgery and chemotherapy, however, 60–80% of these patients will present with recurrent disease between 6 months to 2 years post treatment and the number of responders decreases to ~15% for recurrent cancers.[3] Individuals who succumb to advanced-stage ovarian cancer inevitably become refractory to chemotherapy, resulting in disease progression and death. The source of recurrence and lack of response to chemotherapy is unknown. The focus of this review is to evaluate the question of recurrence and chemoresistance based on the concept of the cancer stem cells.

Cancer Stem Cells

Cancers often arise from normal tissues in the skin, gut and reproductive organs (i.e., ovary, endometrium, breast) where constant turnover is required to ensure a continuous supply of newly differentiated cells. Replacement of the mature cells is accomplished by a highly orchestrated process in which a relatively small population of self-renewing adult stem cells gives rise to progenitor cells, which undergo limited rounds of mitotic division prior to terminal differentiation.[4] In the cancer tissue, this population of long-lived cells with extraordinary expansion potential has been called tumor-initiating cells or cancer stem cells (CSCs).[5,6] CSCs are defined as cells within the tumor that possess the capacity to self-renew and to cause the heterogeneous lineage of cancer cells that comprise the whole tumor.[4,7,8] They were initially identified in leukemia, and more recently in solid tumors.[9-11] Current evidence suggests that these cells are the putative mediators of chemotherapy resistance and tumor progression.[8] It is thought that CSCs are able to survive conventional chemotherapeutic treatments, which usually target fast dividing cells, and give rise to recurrent tumors that are more chemoresistant and more aggressive.[12-14] It is therefore important to identify and characterize these cells to develop new diagnostics and therapeutics.

*Correspondence to: Gil Mor; Email: gil.mor@yale.edu
Submitted: 01/20/11; Accepted: 01/27/11
DOI: 10.4161/cbt.11.8.14967

Ovarian Cancer Cells with Stem-Like Properties (Type I EOC Cells)

One of our earlier observations relating to the heterogeneity of ovarian tumors was associated with the propagation of freshly isolated ovarian cancer cells from the same tumors, but with differential response to chemotherapy. We identified at least two types of EOC cells based on their chemo response: Type I, chemo-resistant and Type II, chemosensitive EOC cells. Further characterization showed that these cells have additional differences in terms of their growth, cytokine production and intracellular markers. While Type II EOC cells represent the "classical" ovarian cancer cells characterized by fast growth and cell division, and lack of cell-to-cell contact inhibition, Type I cells are characterized by slower growth, which is inhibited upon cell-to-cell contact (**Fig. 1**).[10] In addition, Type I, but not Type II EOC cells, have constitutive NFκB activity and constitutively secrete IL6, IL8, MCP-1 and GROα.[14,15] Gene expression microarray analysis obtained from these two types of cells further showed numerous differentially expressed genes including Cytokeratin 18, the TLR adapter protein, MyD88 and several genes that are associated with stemness such as CD44, Oct-4, SSEA-4 and others, which were highly expressed in the Type I EOC cells.[10] These markers were validated at the protein level with western blot analysis (**Table 1**).

These findings suggest that Type I EOC cells may represent the population that has stem-like properties. Indeed, we have demonstrated that Type I EOC cells, as selected by the cell surface marker CD44, are able to form tumors in mice containing both $CD44^+$ and $CD44^-$ cells. More importantly, microscopic analysis of the xenografts obtained showed that Type I EOC cells were able to recapitulate the morphology of the original tumor.[10] Taken together, this suggests that Type I EOC cells can both self-renew and differentiate. The process of differentiation was also observed in vitro wherein 100% of $CD44^+$ cells seeded at very low density eventually gave rise to cultures that looked morphologically different. Whereas the original $CD44^+$ culture doubles every 36 h, the resulting $CD44^-$ culture doubles faster,

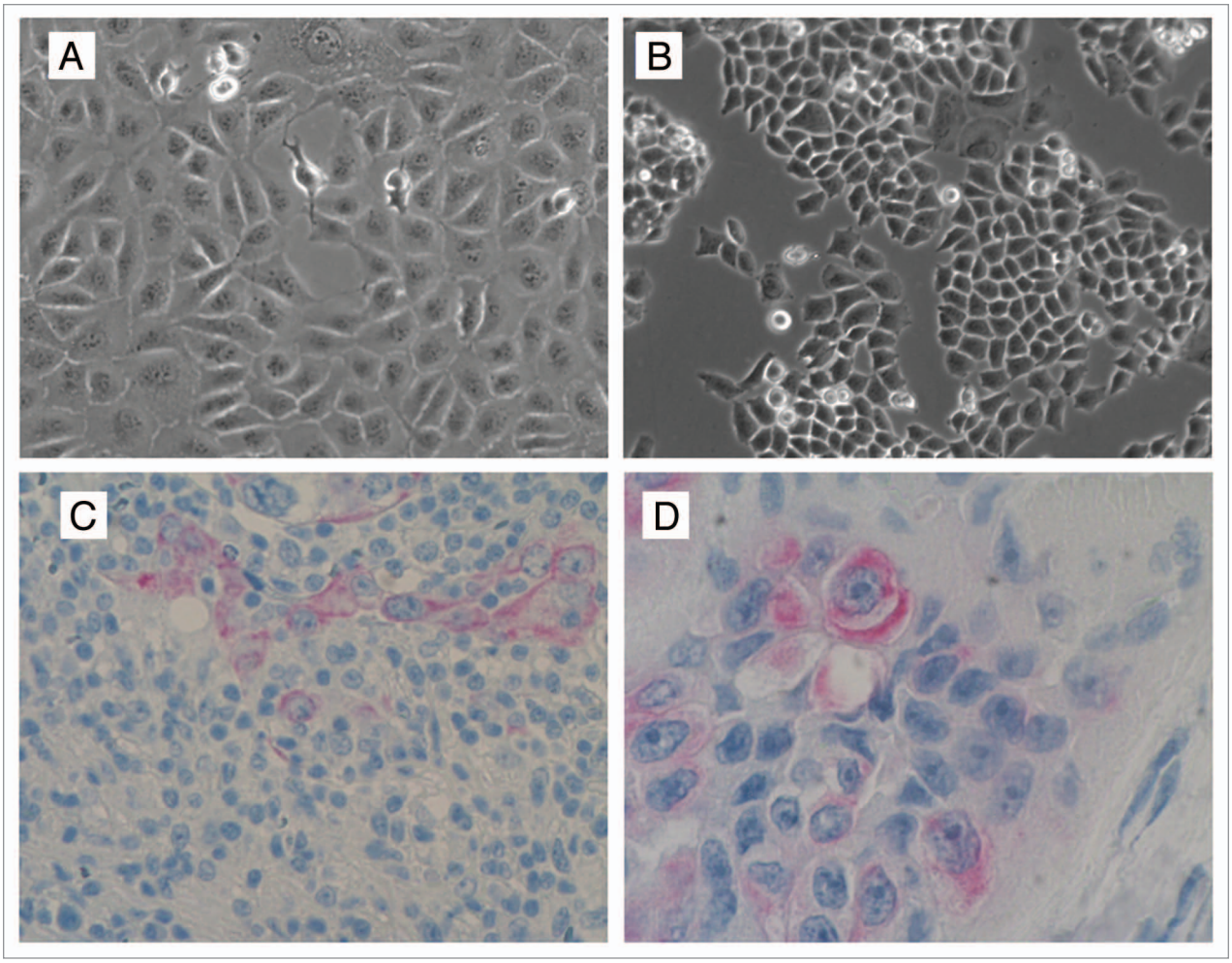


Figure 1. Cellular morphology of ovarian cancer stem cells. Type I EOC stem cells (A) are bigger and have a higher nucleus:cytoplasm ratio compared Type II EOC cells (B); (C and D) Immunostaining of ovarian tumors with Ck18 (pink staining) shows that the morphological difference between the two cell types is maintained in patients with ovarian cancer. $Ck18^+$ Type I EOC stem cells appear larger compared to $Ck18^-$ cells.

every 16 h. Moreover, while $CD44^+$ cells (slow dividing cells) are chemo-resistant, the newly differentiated $CD44^-$ cell cultures (fast dividing cells) became responsive to chemotherapy. Evaluation of the presence of $CD44^+$ cells in ovarian cancer tumors revealed their presence in clusters and in close proximity to the stroma surrounding the tumor. These cells are morphologically less differentiated with an immature appearance including larger size, higher nuclear to cytoplasm (N/C) ratio, vesicular chromatin pattern and prominent nucleoli (**Fig. 1C and D**).

Ovarian Cancer Stem Cells

Bapat et al. reported for the first time the isolation and identification of stem-like cells in ovarian cancer.[16] Using an in vitro model system comprising of 19 spontaneously immortalized clones derived from an advanced-grade patient, the authors were able to show the expression of CD44, EGFR and E-cadherin on 17 of the clones. One of the clones exhibited anchorage-independent growth and formed spheroids. These cells expressed the stem cell factor CD117 and were able to form xenografts in nude mice.

In another study, Zhang et al. reported the isolation of ovarian cancer-initiating cells from primary tumors. Using primary tumor specimens obtained from five different patients, they collected non-adherent cells, which then formed spheroids. The spheroid forming cells were resistant to conventional chemotherapy and formed xenograft tumors of identical phenotype. The main markers identified in these cells were CD117 and CD44.[17]

Still in another study, Deng et al. identified cells with properties of CSCs based on aldehyde dehydrogenase isoform 1 (ALDH1) activity. The study was done mainly with cell lines although ALDH1-positive cells were evaluated in tissue samples from ovarian cancer tumors.[18] ALDH1 could then be an interesting maker for identification of ovarian CSCs but additional validation studies are still needed. A major limitation in this study is the fact that it was limited to the study of cancer cells lines, which carry many variations as a result of culture conditions.

The above studies demonstrated the existence of multiple cell populations in ovarian tumors and showed that the concept of

CSCs is also applicable in ovarian cancer. However, the markers reported have a high degree of variation, which may be associated with the different stages in the hierarchy of the CSCs, or potential difference in origin of the tumors.

In solid tumors, the capacity to form multi-cellular spheroids is one of the most commonly used techniques to evaluate self-renewing properties and stemness potential.[19,20] However, our studies in ovarian cancer suggest that the spheroid-forming cells are one step beyond in the differentiation process and represent the population of Type I EOC cells that underwent epithelial mesenchymal transition (EMT, Yin et al. submitted). Thus, especially in solid tumors, cells obtained form spheroid cultures may already represent a more differentiated population than the original CSCs. Indeed, in our system, many of the stemness markers found in Type I EOC cells are lost in the spheroid cultures. Therefore, it is necessary to consider that when markers identified in spheroid cultures are used to isolate CSCs, it is possible that the epithelial CSCs will be missed.

Self-Renewal and Differentiation

Tissues and organs maintain their original mass and architecture over time through a tightly regulated process of tissue remodeling. As aging or damaged cells undergo apoptosis, new cells will replace them, therefore maintaining the normal tissue structure and function. New evidence suggests that this active process of tissue remodeling is sustained by the adult stem cells. Three major properties are associated with stem cells: (i) differentiation, which is the ability of a stem cell to give rise to a heterogeneous progeny of cells; (ii) self-renewal, which involves the ability to form new identical stem cells; and (iii) homeostatic control or the ability to modulate and balance differentiation and self renewal according to environmental stimuli or genetic control.[21] In normal tissues, stem cells are a minority of the whole organ. Tissue injury is accompanied by the expansion of tissue-specific stem cells through renewal divisions in order to repair the injury. Once the wound is repaired however, the stem cell compartment returns to quiescence.

Many of these characteristics of the normal adult stem cells have also been attributed to the CSCs.[6] Like normal stem cells, most CSCs have been shown to comprise <10% of the tumor tissue. However, our work with ovarian cancer showed a wide range of variation in the percentage of the CSCs. We have identified multiple cases wherein the CSCs, as identified by CD44 staining, comprise more than 50% of the tumor tissue. This shows that the size of the CSC compartment can be highly variable. This observation indicates that the regulation of self-renewal and homeostatic control is altered in Type I EOC stem cells. Therefore, not all characteristics and attributes of normal stem cells are maintained in the CSCs, this is especially true in terms of self-renewal and homeostatic control.

Self-Renewal, Inflammation and Tumor Repair

As described above, in normal tissues following injury, there is an expansion of tissue specific stem cells to initiate repair prior to their differentiation. Once the tissue is repaired, the stem cells return to a quiescent state. In tumor tissue, however, the original expansion following tissue damage (brought by either surgery or chemotherapy) might follow characteristics to those in normal tissues, but the control of the expansion process may be significantly altered and the CSCs may not immediately return to quiescence after repair is completed. This may explain why we observed several tumors from ovarian cancer patients with a CSC compartment comprising more than half of the tumor tissue.

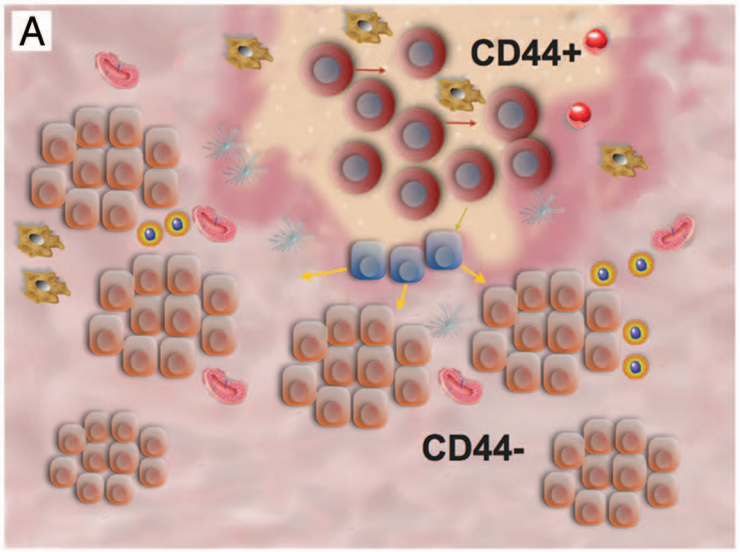
Table 1. Stem cell-associated genes identified by gene expression microarray and validated by real-time PCR or western blot analysis

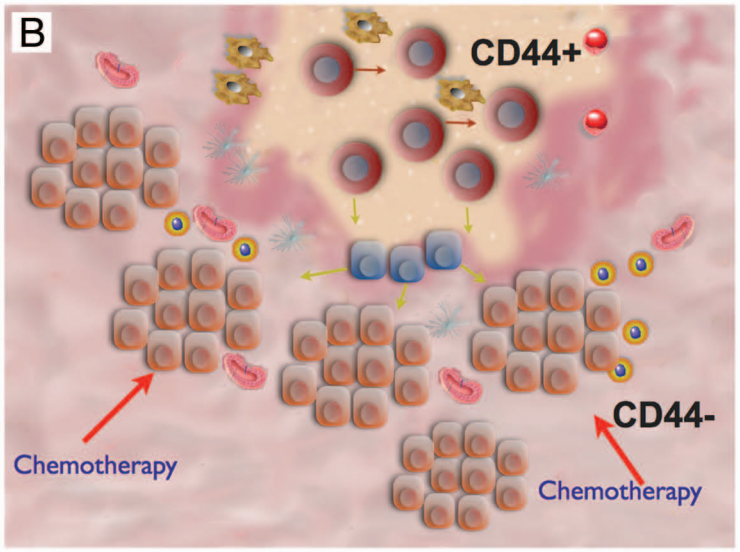
Gene	Type I EOC cells	Type II EOC cells
CD44	+++	-
MyD88	+++	-
Oct4	+++	-
Snail 1	+++	-
Sox 2	+++	-
Klf4	+++	-
IGFBP7	+++	-
RhoE	+++	-
Rac2	+++	-
PML1	+++	-
PML2	+++	-
CK19	+++	-
ALDH1	+++	-
EPCAM	+++	-
Beta-catenin	+++	-
nanog	+++	-
CK-18	+++	-
L1-CAM	+++	-

Using an in vitro wound/healing model, we have shown that the repair process following a scratch wound is driven mainly by the replication or self-renewal of Type I EOC cells. Evaluation of the cytokine profile showed that cultures with a wound had a significant increase in the production of pro-inflammatory cytokines compared to controls without a wound. Interestingly, the cells farthest away from the wound, and not those in the vicinity of the wound, had the highest levels of cytokines. Therefore, we propose that inflammation, as a result of the injury, may affect the process of self-renewal and differentiation. It would be extremely important to determine whether inhibition of inflammation could prevent tumor repair and renewal; if that is the case, then anti-inflammatory compounds could inhibit the repair capacity of EOC stem cells and may have a significant effect on disease recurrence.

Toll-Like Receptor Inflammation, NFκB and Ovarian Cancer Stem Cells

One of the major challenges in understanding the connection between inflammation and cancer is to identify the triggering events that lead to the inflammatory response, the source and





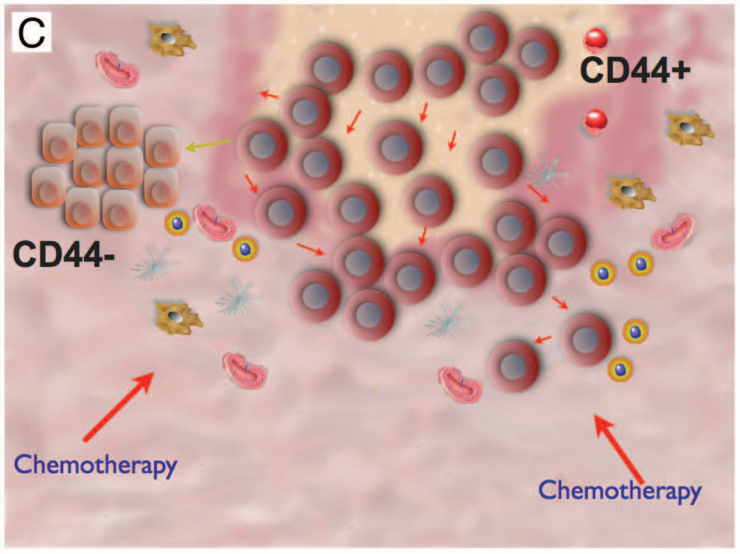


Figure 2. Proposed model depicting complexity of the cellular components of ovarian cancer and the role of the Type I EOC stem cells in chemoresistance and recurrence. (A) We propose that the bulk of ovarian cancer tumors arise from the CD44$^+$ progenitor cancer cells, which have the capacity to self-renew (red arrow) and differentiate (yellow arrow); (B) chemotherapy targets only the CD44$^-$ cancer cells while CD44$^+$ Type I EOC stem cells persist; (C) after treatment, the chemo-resistant CD44$^+$ Type I EOC stem cells, which are capable of maintaining an inflammatory environment, rebuild the tumor mainly by self-renewal than by differentiation; thus, recurrence maybe associated with a larger pool of CD44$^+$ Type I EOC stem cells.

target of the inflammatory signals, and how this can contribute to tumor progression. Toll-like receptors (TLRs) are a family of transmembrane proteins, which recognize and respond to conserved pathogen-associated molecular patterns (PAMPs) that are expressed by microorganisms. To date, ten human TLRs and their specific ligands have been identified. Although TLRs individually respond to limited ligands, collectively as a family, TLRs respond to a wide range of PAMPs associated with bacteria, viruses, fungi and parasites. TLR-2 (with -1 or -6), -4, -5 and -9 recognize mainly bacterial products, while TLR-3 and -8 detect viral components. In addition, some TLRs, such as TLR-2, and -4 respond to endogenous "stress" proteins, such as heat shock protein (Hsp 60), hyaluronan and fibrinogen.[22,23] It should be noted that most of these endogenous ligands are released as part of cellular debris following cell death.[24]

Most TLRs signal through a common pathway since they possess a common intracellular domain known as the Toll/IL-1R homology region (TIR).[25] Following TLR ligation, the TIR recruits the adopter protein MyD88, which then leads to downstream activation of the NFκB and MAP kinase signaling pathways, resulting in an inflammatory response, which is characterized by the production of cytokines and chemokines.[26] TLRs are widely expressed by the cells of the immune system, and initiate an inflammatory process in response to microbial products or stress factors.[27-29] In addition, TLRs have been described in non-immune cells, such as mucosal epithelium and trophoblast cells.[30-32] Similar to immune cells, the ligation of TLRs in non-immune cells results in the expression and secretion of pro-inflammatory cytokines.[33] We found that Type I EOC stem cells, but not Type II EOC cells, have a functional TLR-4 pathway. Indeed, ligation of TLR-4 by LPS or paclitaxel induced cell proliferation and enhanced cytokine/chemokine production in Type I EOC cells.[34]

Recently, Lee et al.[35] reported the expression of TLRs in embryonic stem (ES) cells and showed that TLR ligands stimulate ES cell proliferation and promote differentiation. Since EOC stem cells express a functional TLR pathway, it is plausible that activation of the TLR pathway may enhance the pool of Type I EOC stem cells or promote its differentiation to mature Type II

cells leading to renewal of the tumor. Furthermore, as mentioned above, cellular debris released during surgery or chemotherapy, can be recognized by TLRs expressed in Type I EOC stem cells and this can initiate the tumor repair process responsible for recurrence.

Twist at the Interplay of Self-Renewal, Inflammation and Differentiation

NFκB is one of the key transcription factors in pro-inflammatory responses and copious evidence has been reported that links NFκB activation and cancer development.[36,37] Several cytokines and chemokines produced at the tumor microenvironment by immune cells, such as macrophages, are thought to drive the neoplastic process.[38] However, the contribution of cancer cells themselves in the maintenance of a pro-inflammatory environment that promotes cancer growth is largely overlooked and sometimes considered passive. EOC stem cells, through constitutive cytokine production, may significantly contribute in the maintenance of an inflammatory environment that promotes tissue repair and renewal.[39] The main trigger of constitutive NFκB/cytokine production in EOC stem cells is IKKβ, which is expressed only in Type I EOC stem cells.[15] Upon differentiation, Type I EOC stem cells lose IKKβ expression, NFκB activity, and therefore the capacity to produce cytokines. The regulation of IKKβ during the process of differentiation seems to be regulated by a cluster of micro RNAs, including miR199a and miR214.[15,40]

Evaluation of the genes differentially expressed between Type I and Type II EOC cells revealed that Twist-1, a transcription factor involved in the process of differentiation, is highly expressed in Type II but not expressed in Type I EOC stem cells.[41] Twist-1 is a highly conserved protein that belongs to the family of basic helix-loop-helix (bHLH) proteins.[42-44] Twist-1 has been implicated in the differentiation of multiple cell lineages including muscle, cartilage and osteogenic cells.[45] In mice, Twist-1 was shown to be required for proper development of the head mesenchyme, somites and limb buds.[46,47] Mice lacking Twist-1 die at E10.5, confirming its important role in development and differentiation.[48] In addition, Twist-1 has been shown to be important in the regulation of inflammation and programmed cell death.[43,44] Recent studies have reported Twist-1 expression in several forms of cancer.[49-51] In our studies during the process of in vitro differentiation of Type I EOC cells, Twist-1 levels increased significantly followed by other changes in the cells including decrease in IKKβ, MyD88 and PTEN, and increase in pAKT and IKKα. Further evaluation revealed that Twist-1 is a major regulator of miR199a; and by inducing the expression of miR199a, Twist-1 is able to suppress IKKβ and NFκB activity in the Type I EOC stem cells.[41]

These data suggest that Twist-1 expression has a relevant role not only on the process of differentiation from Type I EOC stem cells to Type II EOC cells,[15,41] but also on the regulation of the inflammatory environment produced and maintained by the CSCs. Therefore, understanding the factors regulating Twist-1 expression is highly important for our understanding of the repair and differentiation processes. Furthermore, it would provide useful information that could allow us to monitor recurrence and prevent metastasis and chemoresistance.

Summary

Recurrence, metastasis and chemoresistance are the major barriers to the successful treatment of ovarian cancer. Chemotherapy eliminates the bulk of the tumor but leaves a core of cancer cells with high capacity for repair and renewal, the ovarian cancer stem cells (**Fig. 2**). In order to improve and advance the management of this disease, it is critical to expand our understanding of the biology of the tumor and its complexity. The identification of ovarian cancer stem cells and its isolation will allow us to improve early detection as well as treatment and prevention.

Acknowledgements

These studies were supported in part by grants from NCI/NIH RO1CA127913, RO1CA118678, The Janet Burros Memorial Foundation, The Sands Family Foundation and the Discovery To Cure Research Program.

References

1. Jemal A, Center MM, Ward E, Thun MJ. Cancer occurrence. Methods Mol Biol 2009; 471:3-29.
2. Schwartz PE. Current diagnosis and treatment modalities for ovarian cancer. Cancer Treat Res 2002; 107:99-118.
3. Jemal A, Ward E, Hao Y, Thun M. Trends in the leading causes of death in the United States 1970–2002. JAMA 2005; 294:1255-9.
4. Clarke MF, Fuller M. Stem cells and cancer: two faces of eve. Cell 2006; 124:1111-5.
5. Paguirigan A, Beebe DJ, Liu B, Alexander C. Mammary stem and progenitor cells: tumour precursors? Eur J Cancer 2006; 42:1225-36.
6. Sell S. Stem cell origin of cancer and differentiation therapy. Crit Rev Oncol Hematol 2004; 51:1-28.
7. Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, et al. Cancer stem cells—perspectives on current status and future directions: AACR Workshop on cancer stem cells. Cancer Res 2006; 66:9339-44.
8. Huang EH, Heidt DG, Li CW, Simeone DM. Cancer stem cells: a new paradigm for understanding tumor progression and therapeutic resistance. Surgery 2007; 141:415-9.
9. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer and cancer stem cells. Nature 2001; 414:105-11.
10. Alvero AB, Chen R, Fu HH, Montagna M, Schwartz PE, Rutherford T, et al. Molecular phenotyping of human ovarian cancer stem cells unravels the mechanisms for repair and chemoresistance. Cell Cycle 2009; 8:158-66.
11. Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer 2008; 8:755-68.
12. Fraser M, Leung B, Jahani-Asl A, Yan X, Thompson WE, Tsang BK. Chemoresistance in human ovarian cancer: the role of apoptotic regulators. Reprod Biol Endocrinol 2003; 1:66.
13. Li J, Sasaki H, Sheng YL, Schneiderman D, Xiao CW, Kotsuji F, et al. Apoptosis and chemoresistance in human ovarian cancer: is Xiap a determinant? Biol Signals Recept 2000; 9:122-30.
14. Silasi DA, Alvero AB, Illuzzi J, Kelly M, Chen R, Fu HH, et al. MyD88 predicts chemoresistance to paclitaxel in epithelial ovarian cancer. Yale J Biol Med 2006; 79:153-63.
15. Chen R, Alvero AB, Silasi DA, Kelly MG, Fest S, Visintin I, et al. Regulation of IKKbeta by miR-199a affects NFkappaB activity in ovarian cancer cells. Oncogene 2008; 27:4712-23.
16. Bapat SA, Mali AM, Koppikar CB, Kurrey NK. Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer. Cancer Res 2005; 65:3025-9.
17. Zhang S, Balch C, Chan MW, Lai HC, Matei D, Schilder JM, et al. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. Cancer Res 2008; 68:4311-20.
18. Deng S, Yang X, Lassus H, Liang S, Kaur S, Ye Q, et al. Distinct expression levels and patterns of stem cell marker, aldehyde dehydrogenase isoform 1 (ALDH1), in human epithelial cancers. PLoS ONE 2010; 5:10277.

19. Yu CC, Chiou GY, Lee YY, Chang YL, Huang PI, Cheng YW, et al. Medulloblastoma-derived tumor stem-like cells acquired resistance to TRAIL-induced apoptosis and radiosensitivity. Childs Nerv Syst 2010; 26:897-904.
20. Leung EL, Fiscus RR, Tung JW, Tin VP, Cheng LC, Sihoe AD, et al. Non-small cell lung cancer cells expressing CD44 are enriched for stem cell-like properties. PLoS One 2010; 5:14062.
21. Zhuge Y, Liu ZJ, Velazquez OC. Adult stem cell differentiation and trafficking and their implications in disease. Adv Exp Med Biol 2010; 695:169-83.
22. Mazzone A, Epistolato MC, De Caterina R, Storti S, Vittorini S, Sbrana S, et al. Neoangiogenesis, T-lymphocyte infiltration and heat shock protein-60 are biological hallmarks of an immunomediated inflammatory process in end-stage calcified aortic valve stenosis. J Am Coll Cardiol 2004; 43:1670.
23. Mazzucato M, Cozzi MR, Pradella P, Perissinotto D, Malmstrom A, Morgelin M, et al. Vascular PG-M/versican variants promote platelet adhesion at low shear rates and cooperate with collagens to induce aggregation. FASEB J 2002; 16:1903-16.
24. Baccala R, Hoebe K, Kono DH, Beutler B, Theofilopoulos AN. TLR-dependent and TLR-independent pathways of type I interferon induction in systemic autoimmunity. Nat Med 2007; 13:543-51.
25. Rock FL, Hardiman G, Timans JC, Kastelein RA, Bazan JF. A family of human receptors structurally related to Drosophila Toll. Proc Natl Acad Sci USA 1998; 95:588-93.
26. McDermott EP, O'Neill LA. Ras participates in the activation of p38 MAPK by interleukin-1 by associating with IRAK, IRAK2, TRAF6 and TAK-1. J Biol Chem 2002; 277:7808-15.
27. Ropert C, Closel M, Chaves AC, Gazzinelli RT. Inhibition of a p38/stress-activated protein kinase-2-dependent phosphatase restores function of IL-1 receptor-associate kinase-1 and reverses Toll-like receptor 2- and 4-dependent tolerance of macrophages. J Immunol 2003; 171:1456-65.
28. Dunne A, O'Neill LA. The interleukin-1 receptor/Toll-like receptor superfamily: signal transduction during inflammation and host defense. Sci STKE 2003; 2003:3.
29. Shoham S, Huang C, Chen JM, Golenbock DT, Levitz SM. Toll-like receptor 4 mediates intracellular signaling without TNFalpha release in response to *Cryptococcus neoformans* polysaccharide capsule. J Immunol 2001; 166:4620-6.
30. Abrahams VM, Bole-Aldo P, Kim YM, Straszewski-Chavez SL, Chaiworapongsa T, Romero R, et al. Divergent trophoblast responses to bacterial products mediated by TLRs. J Immunol 2004; 173:4286-96.
31. Abrahams VM, Mor G. Toll-like receptors and their role in the trophoblast. Placenta 2005; 26:540-7.
32. Sirard JC, Bayardo M, Didierlaurent A. Pathogen-specific TLR signaling in mucosa: mutual contribution of microbial TLR agonists and virulence factors. Eur J Immunol 2006; 36:260-3.
33. Shaykhiev R, Behr J, Bals R. Microbial patterns signaling via Toll-like receptors 2 and 5 contribute to epithelial repair, growth and survival. PLoS One 2008; 3:1393.
34. Kim KH, Xie Y, Tytler EM, Woessner R, Mor G, Alvero AB. KSP inhibitor ARRY-520 as a substitute for Paclitaxel in Type I ovarian cancer cells. J Transl Med 2009; 7:63.
35. Lee SH, Hong B, Sharabi A, Huang XF, Chen SY. Embryonic stem cells and mammary luminal progenitors directly sense and respond to microbial products. Stem Cells 2009; 27:1604-15.
36. Chen R, Alvero AB, Silasi DA, Mor G. Inflammation, cancer and chemoresistance: taking advantage of the toll-like receptor signaling pathway. Am J Reprod Immunol 2007; 57:93-107.
37. Karin M. Nuclear factor kappaB in cancer development and progression. Nature 2006; 441:431-6.
38. Luo JL, Kamata H, Karin M. The anti-death machinery in IKK/NFkappaB signaling. J Clin Immunol 2005; 25:541-50.
39. Kelly MG, Alvero AB, Chen R, Silasi DA, Abrahams VM, Chan S, et al. TLR-4 signaling promotes tumor growth and paclitaxel chemoresistance in ovarian cancer. Cancer Res 2006; 66:3859-68.
40. Chen R, Alvero AB, Silasi DA, Steffensen KD, Mor G. Cancers take their Toll—the function and regulation of Toll-like receptors in cancer cells. Oncogene 2008; 27:225-33.
41. Yin G, Chen R, Alvero AB, Fu HH, Holmberg J, Glackin C, et al. TWISTing stemness, inflammation and proliferation of epithelial ovarian cancer cells through MIR199A2/214. Oncogene 2010; 29:3545-53.
42. Bialek P, Kern B, Yang X, Schrock M, Sosic D, Hong N, et al. A twist code determines the onset of osteoblast differentiation. Dev Cell 2004; 6:423-35.
43. Cheng GZ, Zhang W, Wang LH. Regulation of cancer cell survival, migration and invasion by Twist: AKT2 comes to interplay. Cancer Res 2008; 68:957-60.
44. Cheng GZ, Zhang WZ, Sun M, Wang Q, Coppola D, Mansour M, et al. Twist is transcriptionally induced by activation of STAT3 and mediates STAT3 oncogenic function. J Biol Chem 2008; 283:14665-73.
45. Isenmann S, Arthur A, Zannettino AC, Turner JL, Shi S, Glackin CA, et al. TWIST family of basic Helix-Loop-Helix Transcription Factors Mediate Human Mesenchymal Stromal/Stem Cell Growth and Commitment. Stem Cells 2009; 27:2457-68.
46. Barnes RM, Firulli AB. A twist of insight—the role of Twist-family bHLH factors in development. Int J Dev Biol 2009; 53:909-24.
47. Lee YB, Bantounas I, Lee DY, Phylactou L, Caldwell MA, Uney JB. Twist-1 regulates the miR-199a/214 cluster during development. Nucleic Acids Res 2009; 37:123-8.
48. Baylies MK, Bate M. twist: a myogenic switch in Drosophila. Science 1996; 272:1481-4.
49. Watanabe O, Imamura H, Shimizu T, Kinoshita J, Okabe T, Hirano A, et al. Expression of twist and wnt in human breast cancer. Anticancer Res 2004; 24:3851-6.
50. Puisieux A, Valsesia-Wittmann S, Ansieau S. A twist for survival and cancer progression. Br J Cancer 2006; 94:13-7.
51. Kwok WK, Ling MT, Lee TW, Lau TC, Zhou C, Zhang X, et al. Upregulation of TWIST in prostate cancer and its implication as a therapeutic target. Cancer Res 2005; 65:5153-62.