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Review

Heterogeneity of tumor cells in terms of cancer-initiating cells

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Abstract: Tumors derive from a single cell clone but consist of heterogeneous cell subpopulations with diverse features and functions. A limited number of subclones with a selective advantage can initiate tumors when inoculated into immunocompromised mice, and are called cancer-initiating cells (CICs). CICs can be isolated from the bulk of tumors on the basis of their characteristics, such as high reagent efflux, degradation of reactive oxygen species, and aldehyde dehydrogenase (ALDH) activity. Under normal conditions, new CICs are produced by existing CICs rather than non-CICs. However, under stress conditions, non-CICs can occasionally produce CICs, a phenomenon known as plasticity. The dynamic exchange between CICs and non-CICs may enable tumors to survive under unfavorable conditions. CICs are located in a small portion of tumors. This suggests that microenvironmental factors induce or inhibit the CIC phenotype, which might be regulated by intercellular signaling between tumor cells. This review describes isolation of CICs from tumor cell populations and the microenvironmental factors that regulate CIC phenotypes in uterine cancer and lymphoma. (DOI: 10.1293/tox.2016-0056; J Toxicol Pathol 2017; 30: 1–6)

Key words: cancer initiating cells, aldehyde dehydrogenase, plasticity

Introduction

Tumors derive from a single cell clone but consist of heterogeneous cell subpopulations with diverse features and functions. Such heterogeneity is observed within spatially distinct regions of solid tumors[1–4]. Sequencing analysis has revealed that tumors accumulate mutations, the majority of which do not provide selective benefits, while a small subset of mutations drive cancer development by conferring a selective advantage[3, 4].

A limited number of subclones with a selective advantage survive treatment with anticancer drugs or radiation. Such subclones can initiate tumors when inoculated into immunocompromised mice and are known as cancer stem or cancer-initiating cells (CICs)[5]. CICs can lead to tumor recurrence because they escape apoptosis by effluxing antitumor drugs[6] and degrading reactive oxygen species (ROS)[7], which are related to radiation-induced apoptosis. CICs are generally defined on the basis of their ability to initiate a tumor in a transplantation assay in immunocompromised mice. In leukemia, breast, lung, and colon cancers, the number of CICs is small compared with the total number of tumor cells. However, recent reports reveal that in some tumor types, such as melanomas, the majority of tumor cells can initiate tumors. Therefore, the proportion of CICs varies according to tumor type[8].

Stem cells possess two abilities: self-renewal and multi-differentiation. Asymmetrical division of stem cells yields a stem cell and a more highly differentiated cell. As in the case of physiological stem cells, CICs are derived from CICs but not from non-CICs under normal conditions. However, under some conditions, such as severe hypoxia, non-CICs can yield CICs; this phenomenon is known as "plasticity"[9–12]. The dynamic exchange between CICs and non-CICs may enable tumors to survive under unfavorable conditions. Therefore, CICs may not be a distinct cell type but may be a mode or phenotype of tumor cells.

This review briefly describes isolation of CICs from tumor cell populations and the microenvironmental factors that regulate CIC phenotypes in uterine cancer and lymphoma.

Isolation of CICs

Several methods for isolating CICs have been reported. One is based on the high efflux potential of CICs (Fig. 1A). CICs express high levels of cell membrane-associated ATP-binding cassette (ABC) transporters, which play important roles in efflux of antitumor drugs[6, 13]. When stained with Hoechst 33342 dye, most tumor cells retain the dye and are termed Hoechst 33342-high. However, a small population with high efflux ability eliminates Hoechst 33342 dye and are termed Hoechst 33342-low. Most tumor cells are

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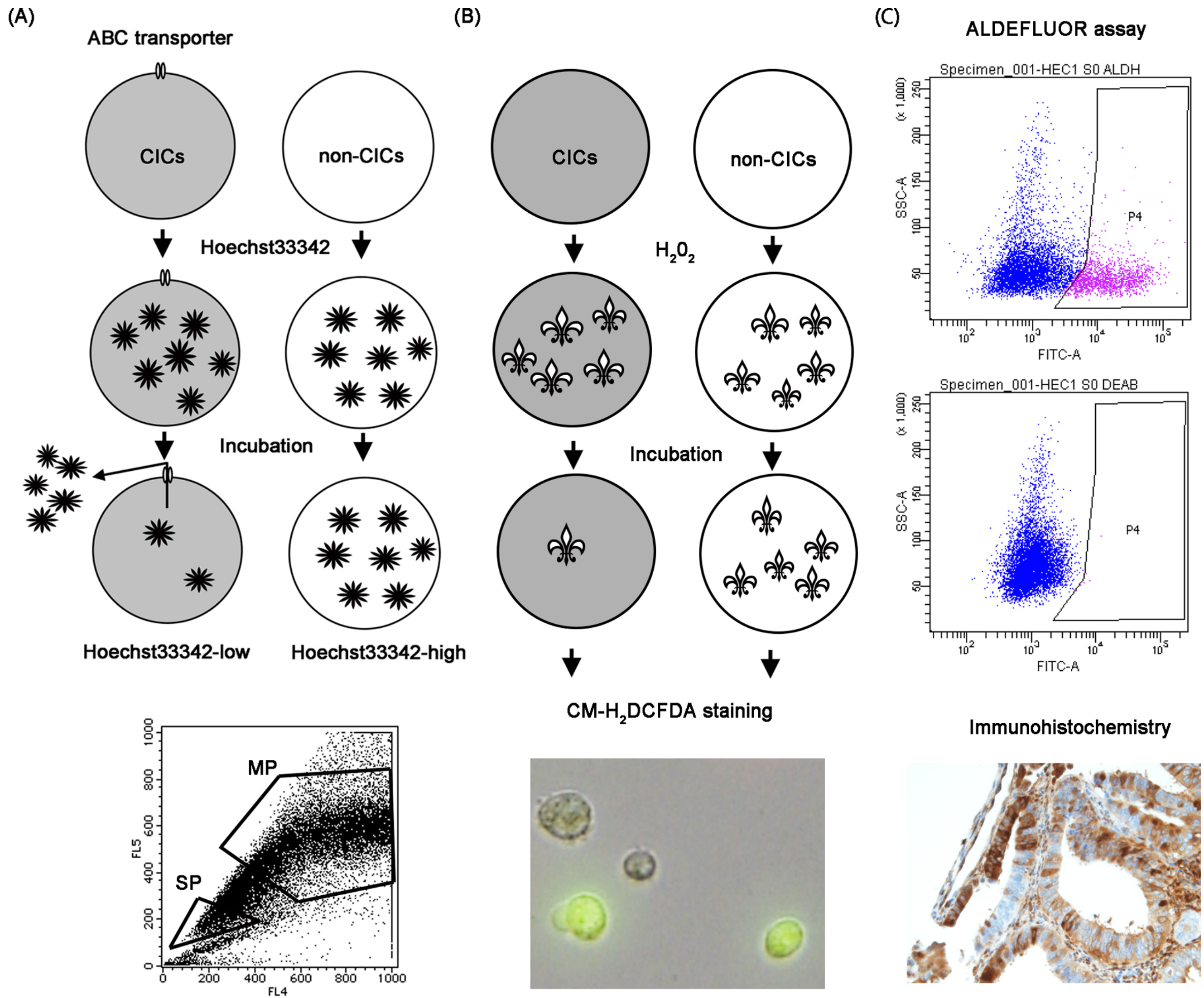

Fig. 1. Isolation of CICs. (A) Isolation of CICs based on the high efflux potential of CICs. CICs express high levels of cell membrane-associated ABC transporters. When stained with Hoechst 33342 dye and incubated for approximately 1 hour, most tumor cells retain the dye (Hoechst 33342-high). In contrast, a small population with high efflux ability eliminates Hoechst 33342 dye (Hoechst 33342-low). The upper part shows an image of Hoechst 33342 staining, in which Hoechst 33342 dye is designated by stars. The lower part shows the dot-blot pattern of flow cytometers, in which Hoechst 33342-high cells are contained in the MP, whereas Hoechst 33342-low cells in the SP. (B) Isolation of CICs with their ability to degrade ROS. CICs express high levels of ROS-degrading enzymes. Upper part shows the image of CM-H_2DCFDA staining, in which ROS is designated as flowers. When treated with hydrogen peroxide, most tumor cells are stained with CM-H_2DCFDA, but a small population remains unstained. The lower part shows the staining of endometrioid carcinoma cells. Green cells are CM-H_2DCFDA-high. (C) Isolation of CICs with ALDH activity. The activity of ALDH is high in CICs. The upper part shows an ALDEFLUOR assay, in which ALDH-high population is figured out in the box (red part). These boxed cells are diminished when treated with N,N-diethylaminobenzaldehyde, the inhibitor of ALDH. The lower part shows the results of immunohistochemical analysis using an anti-ALDH antibody, in which a portion of uterine endometrioid carcinoma cells is ALDH-positive.

Hoechst 33342-high, and are termed the “main population (MP)”, whereas Hoechst 33342-low cells are known as the “side population (SP)”. Flow cytometers equipped with ultraviolet lasers can be used to distinguish the MP and SP, which enable isolation of SP cells. Because SP cells can efflux compounds efficiently, CICs are considered to be involved in the SP. Patrawala *et al.*[14] reported that the SP cells in several tumor cell lines are more tumorigenic than MP cells. This finding is consistent with the concept that CICs are enriched in the SP.

The second method of isolating CICs is based on their ability to degrade ROS (Fig. 1B)[7]. Upon X-ray irradiation, tumor cells produce a large quantity of ROS and undergo apoptosis. CICs express high levels of ROS-degrading enzymes, such as superoxide dismutase, which facilitate their escape from apoptosis by degrading intracellular ROS. Intracellular ROS can be stained with 5-(and 6)-chloromethyl-2’,7’-dichlorohydrofluorescein diacetate (CM-H_2DCFDA).

When treated with hydrogen peroxide, most tumor cells are stained with CM-H_2DCFDA, but a small population remains unstained. These CM-H_2DCFDA-low cells are more tumorigenic than CM-H_2DCFDA-high cells, and CICs are enriched in the CM-H_2DCFDA-low population. The FoxO3a transcription factor regulates expression of ROS-degrading enzymes[15]. In Hodgkin's lymphoma cell lines, most FoxO3a-expressing cells are contained in the CM-H_2DCFDA-low population. A small subpopulation of Hodgkin's lymphoma cells expressing FoxO3a can degrade ROS efficiently and is resistant to apoptosis[16–18]. These FoxO3a-expressing cells may be CICs of Hodgkin's lymphoma. A characteristic of Hodgkin's lymphoma is the presence of giant multinucleated Reed–Sternberg (RS) cells; however, FoxO3a-expressing cells are small mononucleated cells. The L1236 and L428 Hodgkin's lymphoma cell lines include both small mononucleated and giant multinucleated cells. In these cell lines, multinucleated cells are derived from mononucleated cells, but mononucleated cells are not derived from multinucleated cells. The ROS level in mononucleated cells is lower than that in multinucleated cells. These findings suggest that some mononucleated cells, but not RS cells, are the CICs of Hodgkin's lymphoma[16, 17].

The third method of isolation is based on markers of CICs. Several such markers, such as CD133 and CD44 (CD44v isoform), have been reported[19, 20]; aldehyde dehydrogenase (ALDH) is one of the most commonly used. High ALDH activity is present in not only normal stem cells, such as hematopoietic and neural stem cells, but also various types of CICs[21, 22]. Indeed, immunohistochemical analysis using an anti-ALDH antibody revealed that a portion of uterine endometrioid carcinoma cells are ALDH-positive, and cases with high ALDH activity have an unfavorable prognosis (Fig. 1C)[23–25]. ALDH-high cells can be isolated by ALDEFLUOR assay (Fig. 1C). ALDH-high endometrioid carcinoma cells are resistant to apoptosis, mobile, and highly invasive. These findings indicate that ALDH-high cells are similar to CICs[23]. SP and ROS-degrading cells can only be detected while alive. By contrast, ALDH-high cells can be detected immunohistochemically in formalin-fixed paraffin-embedded tissues. Uterine endometrioid carcinoma cells usually express steroid hormone receptors, such as estrogen and progesterone receptors. However, double staining of clinical samples with anti-ALDH and anti-hormone receptor antibodies revealed that ALDH-high cells are negative for these hormone receptors[23]. ALDH-high endometrioid carcinoma cells appear to be immature and negative for "differentiation" markers, such as estrogen and progesterone receptors.

Because ALDH-high cells are readily isolated by flow cytometry, their proteome can be investigated using mass spectroscopy (Table 1). S100A4 is one of the markers isolated by this method[26]. S100A4-knockout cells show reduced proliferation and invasion. These cells also exhibit impaired AKT phosphorylation and matrix metalloproteinase-2 activation, which account for their impaired proliferation and invasion, respectively. Elevated expression of S100A4 is related to myometrial and lymphatic invasion in well- to moderately-differentiated endometrioid carcinoma. Notably, strong and diffuse expression of S100A4 is observed in tumor tissues with a microcystic, elongated, and fragmented ("MELF") pattern, which is associated with a highly invasive phenotype[26]. However, ALDH is not an almighty marker of CICs, because, for example, ALDH is highly expressed in stromal cells, such as dendritic cells and macrophages, in lymphoma[27].

Table 1. List of Proteins Preferentially Expressed in the ALDH-high Population of Uterine Endometrioid Adenocarcinoma Cells[#]

Protein name	Accession number
ALDH1A1	IPI00218914
Neural cell adhesion molecule 1	IPI00435020
Serum deprivation-response protein	IPI00005809
Histone H2B type 1-L	IPI00018534
Nicotinamide N-methyltransferase	IPI00027681
Alkaline phosphatase, tissue-nonspecific isozyme	IPI00419916
Tripartite motif-containing protein 72	IPI00301028
Transcriptional activator protein Pur-beta	IPI00045051
Isoform 1 of Filamin-C	IPI00178352
S100A4	IPI00032313
Tubulin beta-2A chain	IPI00013475

[#]Digest of raw data.

Regulation of ALDH Expression in Tumor Cells

Immunohistochemical analysis indicates that ALDH expression is limited to a small portion of tumors. This suggests the presence of microenvironmental factors that induce or inhibit ALDH expression and suggests that intercellular signaling between tumor cells might regulate ALDH expression. Blocking of TGF-β signaling increases the ALDH-high population[28]. Indeed, phosphorylated Smad-2, an indicator of TGF-β signaling activation, is present at higher levels in ALDH-low than -high endometrioid carcinoma cells. Thus, TGF-β receptor signaling appears to interfere with ALDH expression. TGF-β family members regulate cell fates in developing embryos; for example, Lefty determines the left–right axis. Similarly, the development or differentiation of tumor cells might be controlled by TGF-β family members.

Among the TGF-β receptor ligands, the addition of Nodal to endometrioid carcinoma cells reduces their ALDH expression[28]. Immunohistochemical analysis of clinical samples has revealed Nodal-high tumor cells to be ALDH-low cells, and vice versa. These findings suggest that Nodal inhibits ALDH expression via stimulation of TGF-β signaling in uterine endometrioid carcinoma cells. Intercellular communication mediated by various factors may regulate the "CIC phenotype" of tumor cells and generate heterogeneity.

CICs in Non-Hodgkin's Lymphoma

Lymphomas can be categorized using the surface markers of differentiation status expressed by lymphocytes. For example, peripheral T-cell lymphoma cells express the T-cell marker CD3, and diffuse large B-cell lymphoma cells express the B-cell markers CD79a and CD20. Lymphoplasmacytic lymphoma (LPL) is a rare indolent disease that affects the bone marrow, and is associated with expression of the B- and plasma-cell markers CD20 and CD138[29]. MWCL-1 cells are derived from LPL, and express CD20 and CD138 as surface markers[30]. MWCL-1 cells include three subpopulations: $CD20^-$ $CD138^-$, $CD20^+$ $CD138^-$, and $CD20^+$ $CD138^+$ cells. The first subpopulation expresses neither B- nor plasma-cell markers, the second subpopulation expresses only B-cell markers, and the third subpopulation expresses both B- and plasma-cell markers. When cultured, $CD20^-$ $CD138^-$ cells yield all three subpopulations, but $CD20^+$ cells do not yield $CD20^-$ $CD138^-$ cells (Fig. 2). $CD20^-$ $CD138^-$ cells have higher ROS degradation and in vitro colony formation activities than $CD20^+$ $CD138^-$ and $CD20^+$ $CD138^+$ cells[31]. When cultured in the absence of serum or the presence of an anticancer drug, $CD20^-$ $CD138^-$ cells are resistant to apoptosis, but $CD20^+$ $CD138^+$ cells are not. Immunohistochemical analysis of clinical samples has indicated that LPL tumor cells undergoing apoptosis are $CD138^+$. The production of all three subpopulations, efficient ROS degradation and in vitro colony formation activities, and resistance to apoptosis suggest that $CD20^-$ $CD138^-$ cells are candidate CICs in LPL[31].

As described above, CICs are plastic under stress conditions, in which non-CICs are transformed to CICs. Indeed, hypoxia induces conversion of $CD20^+$ $CD138^-$ cells to $CD20^-$ $CD138^-$ cells, whereas normoxic conditions do not[32]. Among the proteins preferentially expressed by the $CD20^-$ $CD138^-$ subpopulation, expression of the chemokine receptor CXCR7 is elevated under hypoxic conditions. It is possible that the conversion from $CD20^+$ $CD138^-$ to $CD20^-$ $CD138^-$ cells is mediated by signaling via CXCR7 (Fig. 2). This is plausible because the number of $CD20^-$ $CD138^-$ cells increases in a time- and dose-dependent manner when MWCL-1 cells are cultured in the presence of CXCL12, a ligand of CXCR7. In addition, hypoxia enhances the expression of CXCL12 in MWCL-1 cells. Thus hypoxia and the CXCL12-CXCR7 axis appear to be advantageous to $CD20^-$ $CD138^-$ cells. Recently, a constitutively active mutation of CXCR4, a receptor related to CXCR7, has been reported in LPL[33]. CXCR4 shares both the CXCL12 ligand and signal transduction via CXCR4 with CXCR7. CXCR4-mutated LPL exhibits more aggressive behavior than non-mutated LPL. The fact that CICs are resistant to apoptosis and related to aggressive behavior is consistent with the notion that the CICs of LPL utilize the CXCL12-CXCR4/CXCR7 axis.

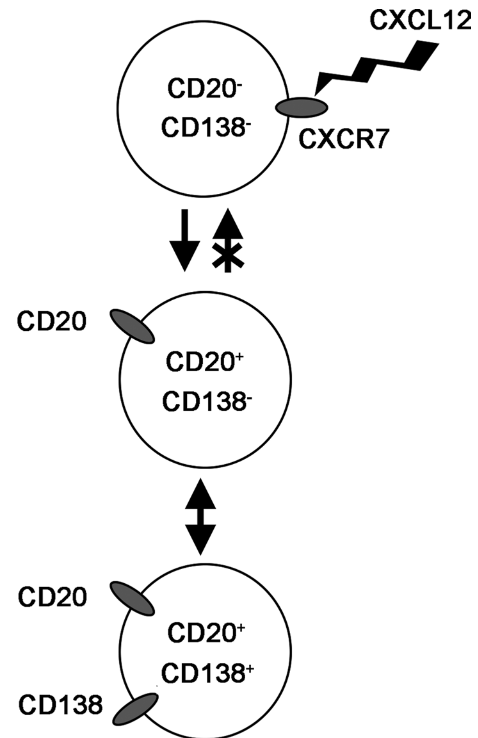

Fig. 2. Relation among three subpopulations of LPL. MWCL-1 cells derived from LPL include three subpopulations: $CD20^-$ $CD138^-$, $CD20^+$ $CD138^-$, and $CD20^+$ $CD138^+$ cells. When cultured, $CD20^-$ $CD138^-$ cells yield all three subpopulations, but $CD20^+$ cells do not yield $CD20^-$ $CD138^-$ cells. $CD20^+$ $CD138^-$ and $CD20^+$ $CD138^+$ cells are interconverted. The $CD20^-$ $CD138^-$ subpopulation expresses the chemokine receptor CXCR7, the ligand of which is CXCL12. The number of $CD20^-$ $CD138^-$ cells increases in a time- and dose-dependent manner when MWCL-1 cells are cultured in the presence of CXCL12.

Future Perspectives

CICs, which are resistant to anticancer drugs and escape from apoptosis, may be a good therapeutic target for cancers. However, they are, under some circumstances, derived from non-CICs due to their plasticity. Therefore, the regulatory mechanism of CICs should be determined. Reagents that block the signals controlling CICs may interfere with their plasticity, leading to their complete elimination. The TGF-β family member Nodal regulates ALDH activity in uterine endometrioid carcinoma, whereas signaling via the chemokine receptor CXCR7 regulates CICs in non-Hodgkin's lymphoma. The regulatory mechanism of CICs thus likely differs among tumor types. Therefore, investigation of the CICs of each tumor type is necessary to facilitate development of novel therapeutics.

Furthermore, assessment of the functions of the factors expressed in CICs is required. ALDH-high tumor cells ex-

press a large number of factors, some of which play roles in invasion and some of which play roles in mobility. Although ALDH-high cells are invasive and mobile, these characteristics are likely mediated by different factors. For example, S100A4 is required for invasion but not mobility. Genome-editing techniques will enable determination of the function of such factors in CICs, which will facilitate development of novel therapeutics.

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References

1. Nowell PC. The clonal evolution of tumor cell populations. Science. **194**: 23–28. 1976. [Medline] [CrossRef]
2. Alpar D, Barber LJ, and Gerlinger M. Chapter 24. Genetic intratumor heterogeneity. In: Epigenetic Cancer Therapy. SG Gray (ed). Elsevier, San Diego. 569–594, 2015.
3. Kandoth C, McLellan MD, Vandin F, Ye K, Niu B, Lu C, Xie M, Zhang Q, McMichael JF, Wyczalkowski MA, Leiserson MD, Miller CA, Welch JS, Walter MJ, Wendl MC, Ley TJ, Wilson RK, Raphael BJ, and Ding L. Mutational landscape and significance across 12 major cancer types. Nature. **502**: 333–339. 2013. [Medline] [CrossRef]
4. Lawrence MS, Stojanov P, Mermel CH, Robinson JT, Garraway LA, Golub TR, Meyerson M, Gabriel SB, Lander ES, and Getz G. Discovery and saturation analysis of cancer genes across 21 tumour types. Nature. **505**: 495–501. 2014. [Medline] [CrossRef]
5. Ravasio R, Ceccacci E, and Minucci S. Self-renewal of tumor cells: epigenetic determinants of the cancer stem cell phenotype. Curr Opin Genet Dev. **36**: 92–99. 2016. [Medline] [CrossRef]
6. Kvinlaug BT, and Huntly BJ. Targeting cancer stem cells. Expert Opin Ther Targets. **11**: 915–927. 2007. [Medline] [CrossRef]
7. Kobayashi CI, and Suda T. Regulation of reactive oxygen species in stem cells and cancer stem cells. J Cell Physiol. **227**: 421–430. 2012. [Medline] [CrossRef]
8. Sugihara E, and Saya H. Complexity of cancer stem cells. Int J Cancer. **132**: 1249–1259. 2013. [Medline] [CrossRef]
9. Meacham CE, and Morrison SJ. Tumour heterogeneity and cancer cell plasticity. Nature. **501**: 328–337. 2013. [Medline] [CrossRef]
10. Kemper K, de Goeje PL, Peeper DS, and van Amerongen R. Phenotype switching: tumor cell plasticity as a resistance mechanism and target for therapy. Cancer Res. **74**: 5937–5941. 2014. [Medline] [CrossRef]
11. Cabrera MC, Hollingsworth RE, and Hurt EM. Cancer stem cell plasticity and tumor hierarchy. World J Stem Cells. **7**: 27–36. 2015. [Medline] [CrossRef]
12. Faurobert E, Bouin AP, and Albiges-Rizo C. Microenvironment, tumor cell plasticity, and cancer. Curr Opin Oncol. **27**: 64–70. 2015. [Medline] [CrossRef]
13. Goodell MA. Multipotential stem cells and 'side population' cells. Cytotherapy. **4**: 507–508. 2002. [Medline] [CrossRef]
14. Patrawala L, Calhoun T, Schneider-Broussard R, Zhou J, Claypool K, and Tang DG. Side population is enriched in tumorigenic, stem-like cancer cells, whereas ABCG2+ and ABCG2− cancer cells are similarly tumorigenic. Cancer Res. **65**: 6207–6219. 2005. [Medline] [CrossRef]
15. Storz P. Forkhead homeobox type O transcription factors in the responses to oxidative stress. Antioxid Redox Signal. **14**: 593–605. 2011. [Medline] [CrossRef]
16. Ikeda J, Mamat S, Tian T, Wang Y, Rahadiani N, Aozasa K, and Morii E. Tumorigenic potential of mononucleated small cells of Hodgkin lymphoma cell lines. Am J Pathol. **177**: 3081–3088. 2010. [Medline] [CrossRef]
17. Ikeda J, Mamat S, Tian T, Wang Y, Luo W, Rahadiani N, Aozasa K, and Morii E. Reactive oxygen species and aldehyde dehydrogenase activity in Hodgkin lymphoma cells. Lab Invest. **92**: 606–614. 2012. [Medline] [CrossRef]
18. Ikeda J, Tian T, Wang Y, Hori Y, Honma K, Wada N, and Morii E. Expression of FoxO3a in clinical cases of malignant lymphoma. Pathol Res Pract. **209**: 716–720. 2013. [Medline] [CrossRef]
19. Grosse-Gehling P, Fargeas CA, Dittfeld C, Garbe Y, Alison MR, Corbeil D, and Kunz-Schughart LA. CD133 as a biomarker for putative cancer stem cells in solid tumours: limitations, problems and challenges. J Pathol. **229**: 355–378. 2013. [Medline] [CrossRef]
20. Nagano O, Okazaki S, and Saya H. Redox regulation in stem-like cancer cells by CD44 variant isoforms. Oncogene. **32**: 5191–5198. 2013. [Medline] [CrossRef]
21. Alison MR, Guppy NJ, Lim SM, and Nicholson LJ. Finding cancer stem cells: are aldehyde dehydrogenases fit for purpose? J Pathol. **222**: 335–344. 2010. [Medline] [CrossRef]
22. Ma I, and Allan AL. The role of human aldehyde dehydrogenase in normal and cancer stem cells. Stem Cell Rev. **7**: 292–306. 2011. [Medline] [CrossRef]
23. Rahadiani N, Ikeda J, Mamat S, Matsuzaki S, Ueda Y, Umehara R, Tian T, Wang Y, Enomoto T, Kimura T, Aozasa K, and Morii E. Expression of aldehyde dehydrogenase 1 (ALDH1) in endometrioid adenocarcinoma and its clinical implications. Cancer Sci. **102**: 903–908. 2011. [Medline] [CrossRef]
24. Mamat S, Ikeda J, Tian T, Wang Y, Luo W, Aozasa K, and Morii E. Transcriptional Regulation of Aldehyde Dehydrogenase 1A1 Gene by Alternative Spliced Forms of Nuclear Factor Y in Tumorigenic Population of Endometrial Adenocarcinoma. Genes Cancer. **2**: 979–984. 2011. [Medline] [CrossRef]
25. Wang Y, Jiang Y, Ikeda J, Tian T, Sato A, Ohtsu H, and Morii E. Roles of histamine on the expression of aldehyde dehydrogenase 1 in endometrioid adenocarcinoma cell line. Cancer Med. **3**: 1126–1135. 2014. [Medline] [CrossRef]
26. Tahara S, Nojima S, Ohshima K, Hori Y, Kurashige M, Wada N, Ikeda J, and Morii E. S100A4 accelerates the proliferation and invasion of endometrioid carcinoma and is associated with the "MELF" pattern. Cancer Sci. **107**: 1345–1352. 2016. [Medline] [CrossRef]
27. Fujita S, Morii E, Rahadiani N, Wada N, Hori Y, Ikeda J, and Aozasa K. Significance of aldehyde dehydrogenase 1 expression in stromal cells of diffuse large B-cell lymphoma. Exp Ther Med. **2**: 591–594. 2011. [Medline]
28. Wang Y, Jiang Y, Tian T, Hori Y, Wada N, Ikeda J, and Morii E. Inhibitory effect of Nodal on the expression of aldehyde dehydrogenase 1 in endometrioid adenocarcinoma

of uterus. Biochem Biophys Res Commun. **440**: 731–736. 2013. [Medline] [CrossRef]

29. Treon SP, Raje N, and Anderson KC. Immunotherapeutic strategies for the treatment of plasma cell malignancies. Semin Oncol. **27**: 598–613. 2000. [Medline]
30. Hodge LS, Novak AJ, Grote DM, Braggio E, Ketterling RP, Manske MK, Price Troska TL, Ziesmer SC, Fonseca R, Witzig TE, Morice WG, Gertz MA, and Ansell SM. Establishment and characterization of a novel Waldenstrom macroglobulinemia cell line, MWCL-1. Blood. **117**: e190–e197. 2011. [Medline] [CrossRef]
31. Wada N, Zhan M, Hori Y, Honma K, Ikeda J, and Morii E. Characterization of subpopulation lacking both B-cell and plasma cell markers in Waldenstrom macroglobulinemia cell line. Lab Invest. **94**: 79–88. 2014. [Medline] [CrossRef]
32. Wada N, Ikeda J, Nojima S, Tahara S, Ohshima K, Okuzaki D, and Morii E. Requirement of CXCL12-CXCR7 signaling for CD20(−) CD138(−) double-negative population in lymphoplasmacytic lymphoma. Lab Invest. **96**: 517–525. 2016. [Medline] [CrossRef]
33. Schmidt J, Federmann B, Schindler N, Steinhilber J, Bonzheim I, Fend F, and Quintanilla-Martinez L. MYD88 L265P and CXCR4 mutations in lymphoplasmacytic lymphoma identify cases with high disease activity. Br J Haematol. **169**: 795–803. 2015. [Medline] [CrossRef]