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Review

Pluripotency transcription factors and cancer stem cells: small genes make a big difference

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Abstract

Cancer stem cells (CSCs) are thought to drive uncontrolled tumor growth, and the existence of CSCs has recently been proven by direct experimental evidence, including tracing cell lineages within a growing tumor. However, CSCs must be analyzed in additional cancer types. Cancer stem cell–like cells (CSCLCs) are a good alternative system for the study of CSCs, which hold great promise for clinical applications. OCT4, NANOG, and SOX2 are three basic transcription factors that are expressed in both CSCLCs and embryonic stem cells (ESCs). These transcription factors play critical roles in maintaining the pluripotence and self-renewal characteristics of CSCLCs and ESCs. In this review, we discuss the aberrant expression, isoforms, and pseudogenes of OCT4, NANOG, and SOX2 in the CSCLC niche, which contribute to the major differences between CSCLCs and ESCs. We also highlight an anticancer therapy that involves killing specific cancer cells directly by repressing the expression of OCT4, NANOG, or SOX2. Importantly, OCT4, NANOG, and SOX2 provide great promise for clinical applications because reducing their expression or blocking the pathways in which they function may inhibit tumor growth and turn-off the cancer "switch." In the future, a clear understanding of transcription factor regulation will be essential for elucidating the roles of OCT4, NANOG, and SOX2 in tumorigenesis, as well as exploring their use for diagnostic and therapeutic purposes.

Key words OCT4, NANOG, SOX2, cancer stem cell, isoforms

Cancer stem cells (CSCs) are a small subset of cells with self-renewal properties that sustain tumor growth and remain in patients after conventional cancer therapy has been completed[1]. CSCs provide clues to the perfect cancer therapy, which may directly turn-off the driver of tumor growth through the use of more powerful and less toxic drugs. In August 2012, *Nature* and *Science* published three papers that represent a paradigm shift in cancer studies[2-4]. In these studies, researchers traced cell lineages within a growing tumor for the first time and discovered CSCs in glioblastomas[2], intestinal adenomas[3], and squamous skin tumors[4]. These studies provided clear and direct experimental evidence to support the hypothesis that some tumors are composed of a minority population of cells that retain both the self-renewal and propagation potentials[1] of the tumor and a vast majority of cells that are merely the nontumorigenic daughter cells of CSCs[5]. This minority population of cells may be referred to as cancer stem cell–like cells (CSCLC) or putative CSCs. The gold standard assay for confirming CSCLCs is the serial transplantation of these cells into an animal model[1]. However, serial transplantation is imperfect because it removes the cells from their natural environment, which may result in changing the behavior of the cells. The new CSC findings require further testing to determine whether these findings also apply to other cancers. CSCLCs have been identified and characterized in brain cancer[6], breast cancer[7], colon cancer[8], ovarian cancer[9], oral squamous cell carcinoma[10], prostate cancer[11], melanoma[12], and many other cancers. However, additional experiments are needed to explain, for example, how CSCs are related to CSCLCs. In principle, CSCLCs have been studied extensively and can be used as a good alternative system for the study of CSCs. Years of cell transplantation studies suggest that the aberrant expression of OCT4, NANOG, and SOX2 isoforms and pseudogenes plays a vital role in tumor transformation, tumorigenesis, and tumor metastasis, but the precise underlying mechanisms are poorly understood. Although CSCLCs and embryonic stem cells (ESCs) share many common characteristics,

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such as unlimited proliferation, studies have suggested that similar mechanisms might be involved in the regulation of both CSCLCs and ESCs[13]. However, there must be something that distinguishes CSCLCs from ESCs. In this review, we compared the different behaviors of OCT4, NANOG, and SOX2 in CSCLCs and ESCs, as well as dissected their influences on CSCLCs and tumor growth. Studies in both CSCLCs and ESCs may influence future cancer treatments.

Transcription Factors and ESCs

OCT4, NANOG, and SOX2 are three pluripotent transcription factors that are well known to contribute to the reprogramming of somatic cells into an ESC-like state. The resulting cells are called induced pluripotent stem cells. The new article "Distinct Lineage Specification Roles for NANOG, OCT4, and SOX2 in Human Embryonic Stem Cells," which was published in *Cell* in April 2012, changed our understanding of the mechanistic functions of OCT4, NANOG, and SOX2 in human ESCs (hESCs)[14]. In hESCs, OCT4, NANOG, and SOX2 function as differentiation repressors, and the positive signals that initiate differentiation are mediated via alternative regulatory pathways. OCT4, NANOG, and SOX2 not only maintain the properties of self-renewal and pluripotency in ESCs but also prevent ESCs from dividing into the wrong cell type in advance. Therefore, each of these transcription factors controls a specific cell fate instead of working as repressors of differentiation. OCT4 controls both extraembryonic and epiblast-derived cell fates that are chosen in a BMP4-dependent manner. Nanog represses embryonic ectoderm differentiation but has little effect on other lineages. SOX2 and SOX3 are redundant and repress mesendoderm differentiation. The overexpression of OCT4, NANOG, and SOX2 does not induce hESC differentiation[1]. Moreover, key LIF and BMP signaling pathways are integrated with the OCT4, NANOG, and SOX2 circuitries through SMAD1 and STAT3[15].

Transcription Factors and CSCs

CSCLCs express many proteins in common with early ESCs, especially OCT4, NANOG, and SOX2. There is a wealth of evidence that the overexpression of these three genes occurs in human malignancies and are relevant to tumor transformation, tumorigenicity, tumor metastasis[16], and distant recurrence after chemoradiotherapy[17]. *OCT4*, *NANOG*, and *SOX2* together have been detected as co–up-regulated in many human cancers, including oral squamous cell carcinoma[10], prostate cancer[18], and breast cancer[16]. OCT4 alone, the most important pluripotent factor, has been shown to be up-regulated in additional cancers, including murine Lewis lung carcinoma[14], human oral squamous cell carcinoma[10], bladder cancer[19], and seminoma cancer[20]. NANOG and SOX2 have been observed to be up-regulated in cases of human somatic tumors. The expression levels of *OCT4*, *NANOG*, and *SOX2* mRNA transcripts, which are detected in tumor cells and CSC niches, are usually higher than those of nontumor tissue or stem cell markers. They are also more frequently overexpressed in poorly differentiated tumors than in well differentiated tumors[16]. In principle, the expression levels of the pluripotent transcription factors should decrease with the differentiation of the cell. However, the mechanistic functions of OCT4, NANOG, and SOX2 in CSCLCs are a little different from their functions in ESCs. Although they both share the property of self-renewal, ESCs emphasize differentiation, whereas CSCs emphasize proliferation. OCT4, NANOG, and SOX2 together maintain the repression of lineage-specific differentiation in hESCs[16]. However, in CSCLCs, the overexpression of *OCT4, NANOG*, and *SOX2* modulates signaling pathways to inhibit apoptosis.

Dysregulation of Transcription Factors and Signaling Pathways

CSCs share several signaling pathways with ESCs[21]. There is evidence that heterogeneous *OCT4, NANOG*, and *SOX2* are involved in signaling pathways related to cell fate determination, proliferation, and apoptosis in cancer cells. Molecular mechanisms that regulate stem cell self-renewal in the early embryo may be re-activated during the dysregulated proliferation observed in tumorigenesis[22]. OCT4 is reported to maintain the survival of CSCLCs partly by inhibiting apoptosis through the OCT4/TCL1/AKT1 pathway. TCL1 is transcriptionally controlled by OCT4 and enhances the kinase activity of AKT1, whose activation could promote cell proliferation[23]. When *OCT4* is up-regulated, the Oct4/Tcl1/Akt1 pathway is activated, which contributes to inhibiting tumor cell apoptosis. Meanwhile, *SOX2* participates in the SOX2/ORAIL/STIM1 pathway. Store-operated Ca^{2+} entry (SOCE) plays an important role in a variety of physiologic and pathophysiologic processes, including apoptosis. Reduced SOCE is one of the factors that contribute to the anti-apoptotic milieu of prostate cancer[24]. The key components of SOCE are ORAIL1 and STIM1. ORAIL1 works as a gatekeeper to the plasma membrane, whereas STIM1 senses the depletion of the endoplasmic reticulum Ca^{2+} store before interacting with ORAIL1 to allow SOCE[17,24]. The up-regulation of *SOX2* reduces the expression of ORAIL1, thus reducing SOCE. *NANOG* is a direct target of the LIF-STAT3 pathway, and it also maintains self-renewal of CSCs through the IGF1R signaling pathway[25]. *NANOG* overexpression enhances the expression of many CSC-associated molecules, such as CD133, ABCG2, ALDH1A1, and CD44[26]. The Hedgehog (Hh) and Notch signaling pathways also take part in cell fate determination and regulate tumorigenicity. *NANOG* overexpression was found to have a prominent effect on gynecologic tumorigenesis, whereas the dysregulation of *OCT4* and *SOX2* may vary in a context-dependent manner[27]. Different cancers involve different signaling pathways, but it seems that *OCT4*, *NANOG*, and *SOX2* are nearly always up-regulated to activate or repress the previously discussed cancer-related pathways.

Isoforms and Pseudogenes of Transcription Factors

The molecular weight of OCT4 in bladder tumor cells is

slightly higher than that in NT2 cells, possibly due to a differential posttranslational modification of *OCT4*[3]. Despite the dysregulation of *OCT4*, *NANOG*, and *SOX2* in signaling pathways, the expression of their isoforms and pseudogenes may contribute to their different behaviors in ESCs and CSCs. Due to the high homology between *OCT4*, *NANOG*, and *SOX2*, it may be possible that their isoforms, pseudogenes, or mutations function in the self-renewal process of CSCs.

The human *OCT4* gene can generate at least 3 transcripts (OCT4A, OCT4B, and OCT4B1) and 4 protein isoforms (OCT4A, OCT4B-190, OCT4B-265, and OCT4B-164) through alternative splicing and alternative translation initiation[14,24]. OCT4A is highly expressed in hESCs and regulates the self-renewal of pluripotent cells. OCT4B is expressed at low levels in both pluripotent and nonpluripotent cells and lacks the stemness-promoting characteristics of OCT4A[28]. Furthermore, OCT4A is more abundant in hESCs than in OCT4B. Additionally, OCT4A is nuclear, whereas OCT4B is cytoplasmic[29]. In a recent study, OCT4B was shown to be expressed in 42 somatic tumor cell lines, whereas OCT4A was not expressed in the tumor cell lines examined[30]. In summary, OCT4A possesses the unique function of maintaining self-renewal in ESCs. Although OCT4B is not sufficient to maintain stem cell self-renewal or an undifferentiated state, it would be of interest to determine whether OCT4B can cooperate with OCT4A to transform nontumorigenic cells[29]. (mRNAs) *OCT4A* and *OCT4B* encode proteins that share a POU DNA-binding domain and C-terminal domain but differ in sequence at the N termini. Thus, OCT4A functions as a transcriptional activator, whereas OCT4B does not function in this manner[29].

Among the 11 *NANOG* pseudogenes, *NANOGP1* is transcribed in different leukemic cells, and it is hypothesized that the transcriptional activation of NANOGP1 represents a "gain-of-stem cell function" in acute leukemia[31]. *NANOGP8*, a retrogene localized in the nuclei of transfected cells[13], is transcribed in 5 tumor cell lines, 2 teratocarcinoma cell lines, and 3 tumor tissues[13]. NANOG was also identified in selected tumor cell lines[32], and studies have shown that NANOG can play a crucial role in maintaining the self-renewal of CSCs through the IGF1R signaling pathway[25]. However, previous studies have shown compelling evidence that (mRNA) *NANOG* in cancer cells is derived predominantly from *NANOGP8*[33]. *NANOGP8* is the dominant gene in CSCs[13], whereas *NANOG* is specifically expressed in ESCs, germ lineage cells[34], and neonatal human fibroblasts[32]. *NANOGP8* differs from *Nanog* by only 3 amino acids. The mechanisms underlying *NANOG* and *NANOGP8* regulation in ESCs and CSCs requires further investigation. One potential molecular mechanism is that both NANOG and NANOGP8 function as transcription factors in a cell type-specific manner[32].

SOX2 has no known isoforms to date, but *SOX2* gene mutations are associated with several human diseases, such as anopthalmia, optic nerve hypoplasia, and other ocular disorders. Despite the critical role of *OCT4*, *NANOG*, and *SOX2* (including their isoforms) in CSCs, detailed knowledge of their expression patterns, functions, and precise regulatory mechanisms in CSCLCs and tumor growth are lacking. Multiple assays, including marker analysis, loss-of-function assays, gain-of-function assays, and analyses of genetic and epigenetic signatures, together with simple approaches and methods used to detect and distinguish *OCT4*, *NANOG*, and *SOX2* isoforms, must be refined and performed in the future to address these issues.

Transcription Factors and Cancer Treatment

OCT4, NANOG, and SOX2 exhibit a vast clinical potential. They can serve as valuable markers of tumorigenesis[16] and act as molecular switches that control the CSC cell fate during cancer development[14]. OCT4 is a useful marker of germ cell metastasis in extragonadal tissues[35], and can also be regarded as a new potential molecular marker in bladder tumors[18]. The overexpression of NANOG predicts tumor progression and poor prognosis in colorectal cancer[36]. In addition, NANOG is associated with tumor development[37]. NANOG/NANOGP8 expression is associated with the early developmental stages of gastric carcinogenesis[22]. Moreover, extensive loss-of-function analysis reveals that the RNA interference-mediated Nanog knockdown inhibits tumor development[33]. SOX2 can potentially be used as a pathologic marker to distinguish tumor from non-tumor in prostate tissues and to predict the prognosis of prostate cancer[38]. SOX2 overexpression induces a proximal phenotype in the distal airways/alveoli and promotes lung cancer[39]. Studies have shown that the expression of both OCT4 and NANOG can be important prognostic markers for oral cancer. The expression of NANOG alone is a good survival prognosis marker in oral squamous cell carcinoma patients. Importantly, there is significant associations between OCT4 expression, NANOG expression, cancer stage and patient survival[10]. OCT4 and SOX2 are related to rectal cancer and are associated with distant recurrence after chemoradiotherapy[40]. There is solid evidence that OCT4, NANOG, and SOX2 can contribute to cancer treatment. Further work should focus on functional analysis to define the roles of these transcription factors in determining the CSC phenotypes, revealing the precise regulatory mechanisms and identifying new components of the transcriptional regulatory networks that may be relevant to tumor transformation, tumorigenesis, and metastasis. Tumors may be controlled by restricting the expression of OCT4, NANOG, and SOX2 or by disrupting the molecular pathways that are altered in CSCs.

A View to the Future

The existence of CSCs is established. In the future, the field of CSC research will certainly be in the spotlight. Eliminating cancer cells with the potential for self-renewal and tumor propagation should be the target of cancer drug development. It is also important to discriminate CSCs from normal stem cells in cancer treatment, which will require the identification of drug targets unique to CSCs[5]. Moreover, CSCs are particularly resistant to conventional chemotherapy and radiotherapy compared with non-CSCs[41]. Previous studies have demonstrated the important contribution of

pluripotent transcription factors to CSC function. The genome-wide mapping of *OCT4*, *NANOG*, and *SOX2* has revealed that these proteins co-target multiple downstream genes[15] associated with many chromatin-associated activities and complexes[42], and these proteins form a regulatory network to control cell phenotypes. *OCT4*, *NANOG*, and *SOX2* may fulfill the criteria for CSC-specific agents because their expression levels are low or absent in normal stem cells; they are also the genes responsible for maintaining the signature features of CSCs, therefore, drugs targeting their activity are sure to have good efficacy. However, our understanding of the molecular structure and mechanism of these pluripotent transcription factors is still in its infancy. Future studies of OCT4, NANOG, and SOX2 are needed to reveal the underlying mechanisms of tumorigenesis and to design individualized therapies for cancer patients.

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References

[1] Clarke M, Dick J, Dirks P, et al. Cancer stem cells—perspectives on current status and future directions: AACR Workshop on cancer stem cells. Cancer Res, 2006,66:9339–9344.

[2] Chen J, Li Y, Yu TS, et al. A restricted cell population propagates glioblastoma growth after chemotherapy. Nature, 2012,488:522–526.

[3] Schepers AG, Snippert HJ, Stange DE, et al. Lineage tracing reveals Lgr5$^+$ stem cell activity in mouse intestinal adenomas. Science, 2012,337:730–735.

[4] Driessens G, Beck B, Caauwe A, et al. Defining the mode of tumour growth by clonal analysis. Nature, 2012,488:527–530.

[5] Bernstein A. Cancer stem cells: the centrality of translational research to cancer control. CMAJ, 2007,176:29–30.

[6] Singh SK, Clarke ID, Terasaki M, et al. Identification of a cancer stem cell in human brain tumors. Cancer Res, 2003,63:5821–5828.

[7] Al-Hajj M, Wicha MS, Benito-Hernandez A, et al. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A, 2003,100:3983–3988.

[8] O'Brien CA, Pollett A, Gallinger S, et al. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature, 2007,445:106–110.

[9] Zhang S, Balch C, Chan MW, et al. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. Cancer Res, 2008,68:4311–4320.

[10] Chiou SH, Yu CC, Huang CY, et al. Positive correlations of Oct-4 and Nanog in oral cancer stem-like cells and high-grade oral squamous cell carcinoma. Clin Cancer Res, 2008,14:4085–4095.

[11] Maitland NJ, Collins AT. Prostate cancer stem cells: a new target for therapy. J Clin Oncol, 2008,26:2862–2870.

[12] Schatton T, Murphy GF, Frank NY, et al. Identification of cells initiating human melanomas. Nature, 2008,451:345–349.

[13] Zhang J, Wang X, Li M, et al. NANOGP8 is a retrogene expressed in cancers. FEBS J, 2006,273:1723–1730.

[14] Wang X, Dai J. Concise review: isoforms of OCT4 contribute to the confusing diversity in stem cell biology. Stem Cells, 2010,28:885–893.

[15] Chen X, Xu H, Yuan P, et al. Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. Cell, 2008,133:1106–1117.

[16] Ben-Porath I, Thomson MW, Carey VJ, et al. An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. Nat Genet, 2008,40:499–507.

[17] Feske S. Calcium signalling in lymphocyte activation and disease. Nat Rev Immunol, 2007,7:690–702.

[18] Gu G, Yuan J, Wills M, et al. Prostate cancer cells with stem cell characteristics reconstitute the original human tumor in vivo. Cancer Res, 2007,67:4807–4815.

[19] Atlasi Y, Mowla SJ, Ziaee SA, et al. Oct-4, an embryonic stem cell marker, is highly expressed in bladder cancer. Int J Cancer, 2007,120:1598–1602.

[20] Ezeh UI, Turek PJ, Reijo RA, et al. Human embryonic stem cell genes OCT4, NANOG, STELLAR, and GDF3 are expressed in both seminoma and breast carcinoma. Cancer, 2005,104:2255–2265.

[21] Boyer LA, Lee TI, Cole MF, et al. Core transcriptional regulatory circuitry in human embryonic stem cells. Cell, 2005,122:947–956.

[22] Hart AH, Hartley L, Ibrahim M, et al. Identification, cloning and expression analysis of the pluripotency promoting Nanog genes in mouse and human. Dev Dyn, 2004,230:187–198.

[23] Mirza AM, Gysin S, Malek N, et al. Cooperative regulation of the cell division cycle by the protein kinases RAF and AKT. Mol Cell Biol, 2004,24:10868–10881.

[24] Takeda J, Seino S, Bell GI. Human Oct3 gene family: cDNA sequences, alternative splicing, gene organization, chromosomal location, and expression at low levels in adult tissues. Nucleic Acids Res, 1992,20:4613–4620.

[25] Shan J, Shen J, Liu L, et al. Nanog regulates self-renewal of cancer stem cells through the insulin-like growth factor pathway in human hepatocellular carcinoma. Hepatology, 2012,56:1004–1014.

[26] Jeter CR, Liu B, Liu X, et al. Nanog promotes cancer stem cell characteristics and prostate cancer resistance to androgen deprivation. Oncogene, 2011,30:3833–3845.

[27] Mak VC, Siu MK, Wong OG, et al. Dysregulated stemness-related genes in gynecological malignancies. Histol Histopathol, 2012,27:1121–1130.

[28] Gao Y, Wang X, Han J, et al. The novel OCT4 spliced variant OCT4B1 can generate three protein isoforms by alternative splicing into OCT4B. J Genet Genomics, 2010,37:461–465.

[29] Lee J, Kim HK, Rho JY, et al. The human OCT-4 isoforms differ in

their ability to confer self-renewal. J Biol Chem, 2006,281:33554–33565.
[30] Mueller T, Luetzkendorf J, Nerger K, et al. Analysis of OCT4 expression in an extended panel of human tumor cell lines from multiple entities and in human mesenchymal stem cells. Cell Mol Life Sci, 2009,66:495–503.
[31] Eberle I, Pless B, Braun M, et al. Transcriptional properties of human NANOG1 and NANOG2 in acute leukemic cells. Nucleic Acids Res, 2010,38:5384–5395.
[32] Ambady S, Malcuit C, Kashpur O, et al. Expression of NANOG and NANOGP8 in a variety of undifferentiated and differentiated human cells. Int J Dev Biol, 2010,54:1743–1754.
[33] Jeter CR, Badeaux M, Choy G, et al. Functional evidence that the self–renewal gene NANOG regulates human tumor development. Stem Cells, 2009,27:993–1005.
[34] Rodda DJ, Chew JL, Lim LH, et al. Transcriptional regulation of nanog by OCT4 and SOX2. J Biol Chem, 2005,280:24731–24737.
[35] Cheng L. Establishing a germ cell origin for metastatic tumors using OCT4 immunohistochemistry. Cancer, 2004,101:2006–2010.
[36] Meng HM, Zheng P, Wang XY, et al. Overexpression of nanog predicts tumor progression and poor prognosis in colorectal cancer. Cancer Biol Ther, 2010,9.
[37] Zhang J, Wang X, Chen B, et al. The human pluripotency gene NANOG/NANOGP8 is expressed in gastric cancer and associated with tumor development. Oncol Lett, 2010,1:457–463.
[38] Jia X, Li X, Xu Y, et al. SOX2 promotes tumorigenesis and increases the anti-apoptotic property of human prostate cancer cell. J Mol Cell Biol, 2011,3:230–238.
[39] Lu Y, Futtner C, Rock JR, et al. Evidence that SOX2 overexpression is oncogenic in the lung. PLoS One, 2010,5:e11022.
[40] Saigusa S, Tanaka K, Toiyama Y, et al. Correlation of CD133, OCT4, and SOX2 in rectal cancer and their association with distant recurrence after chemoradiotherapy. Ann Surg Oncol, 2009,16:3488–3498.
[41] Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature, 2006,444:756–760.
[42] Orkin SH, Hochedlinger K. Chromatin connections to pluripotency and cellular reprogramming. Cell, 2011,145:835–850.