IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 20, Issue 2 Ser. 2 (Mar. – Apr. 2025), PP 41-51
www.Iosrjournals.Org

Research Progress On The Involvement Of Oct4 In Regulating The Fate Of Cancer Stem Cell

Zhigang Hu, Xiang Cao, Fanjun Zeng

Department Of Respiratory And Critical Care Medicine, The First College Of Clinical Medicine Science, China Three Gorges University, Yichang 443003, People's Republic Of China
Department Of Respiratory And Critical Care Medicine, Yichang Central People's Hospital, Yichang 443003, People's Republic Of China
Clinical Medical Research Center For Precision Diagnosis And Treatment Of Lung Cancer And Management Of Advance Cancer Pain Of Hubei Province

Abstract:
Cancer remains a leading cause of mortality worldwide, with increasing incidence and challenges in treatment resistance and recurrence. Cancer stem cells (CSCs), a rare subpopulation within tumors, drive tumor initiation, metastasis, and therapy resistance. Octamer-binding transcription factor 4 (Oct4), a key regulator of stemness, plays a pivotal role in maintaining CSC self-renewal, pluripotency, and survival. This review summarizes current research on Oct4's mechanisms in regulating CSC fate, including self-renewal via Wnt/β-catenin and TGF-β pathways, differentiation through lineage-specific markers, proliferation via PI3K/AKT and survivin activation, apoptosis resistance by modulating caspase and Bcl-2 family proteins, and autophagy-mediated stress adaptation. Oct4 also contributes to CSC drug resistance and radioresistance, impacting clinical outcomes. Targeting Oct4 and its associated pathways may offer novel therapeutic strategies to eradicate CSCs and improve cancer treatment. Further studies are needed to elucidate Oct4's precise regulatory networks in CSCs for potential clinical applications.

Keywords: *Oct4, cancer stem cells, self-renewal, differentiation, apoptosis, autophagy, therapeutic resistance.*

Date of Submission: 14-04-2025 Date of Acceptance: 24-04-2025

I. Introduction

According to domestic data statistics, in 2015, approximately 4,292,000 new cancer cases were reported in China, and about 2,814,000 individuals succumbed to cancer [1]. With the continuous growth of the population and the intensification of aging, the prevalence and diagnosis rates of tumors are inevitably on the rise. Despite the gradual decline in tumor-related mortality compared to the past, the number of deaths due to tumors is still significantly increasing because of the escalating incidence. Moreover, due to factors such as insufficient awareness, economic considerations, and psychological aspects, the 5-year survival rate of tumor patients in China is less than 37%, lagging far behind that of the United States and other developed countries [1].

In recent years, with the exploration of various treatment modalities such as radiotherapy, chemotherapy, molecular targeted therapy, and immunotherapy, the survival time of tumor patients has improved compared to earlier times. However, many patients still face challenges such as tumor drug resistance, recurrence, distant

metastasis, and other detrimental factors. The proposition of the "tumor stem cell theory" offers a glimmer of hope for the early detection and thorough treatment of cancer. As a core transcription factor that regulates and maintains the characteristics of stem cells, Oct4 plays a crucial role in the self-renewal, proliferation, differentiation, and apoptosis of cancer stem cell. This article presents a concise overview of the mechanisms and research advancements of the Oct4 gene in regulating the fate of cancer stem cell.

II. Cancer Stem Cell And Oct4

Cancer stem cell are regarded as a small subset of cells within tumor tissues that possess capabilities such as self-renewal, continuous proliferation, and multi-potential differentiation. Despite constituting only 0.01% to 1% of the tumor population, they play a vital role in tumor initiation, drug resistance, metastasis, recurrence, and radiation resistance [2]. Patrawala et al. summarized the following characteristics of cancer stem cell: (1) The proportion of cancer stem cell within tumor tissues is typically very low; (2) Despite having a strong proliferation potential, cancer stem cell are largely in a quiescent state in the normal microenvironment; (3) cancer stem cell can achieve self-renewal and differentiate into various cell types; (4) cancer stem cell can specifically express stem cell markers or biological features to facilitate identification and enrichment [3]. To better investigate the role of cancer stem cell in the genesis and development of tumors, the separation, identification, and cultivation of cancer stem cell have become of paramount importance.

In the 1990s, significant breakthroughs were made in the research on cancer stem cell. Researchers initially isolated a leukemia cell population with surface markers CD34+CD38- from the blood of patients with acute myeloid leukemia [4]. Subsequently, scientists inoculated various leukemia cells into NOD/SCID mice and discovered that only the CD34+CD38- Thy-1 cell population could form tumor cells within the mice, thereby confirming their status as leukemia cancer stem cell. Al-Hajj et al. were the first to confirm the existence of cancer stem cell in solid tumors. They employed flow cytometry to screen out tumor cells specifically expressing CD44+CD24- and conducted nude mouse inoculation and tumor formation experiments [5]. Only 100 cells expressing CD44+CD24- were capable of forming tumors in NOD/SCID mice, while unclassified cells required 5×10^4 cells to establish tumors. Later, researchers utilized the isolation and culture methods of neural stem cells to separate and identify a tumor cell population with surface markers CD133+ from brain tumor surgical specimens, confirming their possession of characteristics such as multi-directional differentiation, proliferation, and self-renewal typical of neural stem cells [6]. When 100 CD133+ tumor cells were inoculated into the brains of NOD/SCID mice, they could form xenograft tumors. The newly formed tumors shared the same composition as the primary tumors, expressing neural nestin antigen, CD133+ antigen, and other similar characteristics. In contrast, inoculating 105 CD133-negative tumor cells failed to induce tumor formation in the mouse brains. Eramo et al. utilized flow cytometry to perform aseptic cell sorting on fresh lung cancer tissues and cultivated them in serum-free medium. They found that only the CD133+ cell population could form tumor stem cell spheres, which could give rise to metastatic tumors upon inoculation into mice [7]. Subsequently, CD133+ tumor stem cell populations were also identified in liver cancer and colon cancer, and they all exhibited the characteristics of stem cells such as self-renewal, proliferation, and multi-directional differentiation potential [7,8]. In other tumors, such as prostate cancer, gastric cancer, melanoma, retinoblastoma, head and neck tumors, and pancreatic cancer, cancer stem cell with specific antigen expression have also been detected [9-13]. Additionally, Goodell et al. isolated a small subset of stem cell-rich cell populations from mouse bone marrow and designated them as side population cells. These cells have the ability to expel the fluorescent dye Hoechst 33342 from the cell nucleus, and this phenomenon is also regarded as one of the characteristics of stem cells [14]. Similar phenomena have also been observed in the study of cancer stem cell. Ho et al. employed flow cytometry to sort side population cells from six human lung cancer cell lines

(A549, H23, H460, H2170, HTB-58, H441). Compared with non-side population cells, side population cells exhibited a higher probability of chemotherapy resistance and a stronger capacity to form subcutaneous tumors in nude mice [15]. Through the aforementioned methods, researchers have essentially completed the isolation, identification, and culture of cancer stem cell. The subsequent research direction focuses on the mechanisms by which cancer stem cell regulate self-renewal, proliferation, multi-directional differentiation, apoptosis, autophagy, and other fates, as well as the pathways through which they develop bacterial resistance, radioresistance, distant metastasis, and recurrence. Through studies on embryonic stem cells, pluripotent stem cells, and cell reprogramming, the octamer-binding transcription factor 4 (Octamer-binding transcription factor 4, Oct-4) has gradually come into the purview of researchers.

The Oct4 gene, also known as Oct3/4 or pou5f1, is located on human chromosome 6p21.3, spanning approximately 7 kb and containing five exons. It is one of the significant transcription factors within the POU family [16]. The protein encoded by the Oct4 gene can be roughly divided into three components: the C-terminal, the middle POU DNA binding domain, and the N-terminal [17, 18]. The C-terminal and the middle POU DNA binding domain are relatively independent. Based on the variations in the proteins encoded by the N-terminal, Oct4 can be approximately classified into three subtypes: Oct4-A, Oct4-B, and Oct4B1 [19]. Oct4-A is localized within the cell nucleus and possesses the activities of self-renewal and maintenance of undifferentiated potential, while Oct4-B is located in the cytoplasm and lacks these two functionalities. Consequently, current research primarily focuses on the Oct4-A subtype [18]. It is notable that there are eight pseudogenes of Oct4, among which six are pseudogenes of Oct4-A [20, 21]. Pseudogenes and Oct4A exhibit high homology, but typically lack intron sequences and promoter sequences for gene transcription, and thus do not possess self-renewal activity [18]. Zhou et al. transfected Oct4 pseudogenes into U251 cells via plasmid transfection and inoculated them into mice. U251 cells did not form tumors in mice, indicating that pseudogenes lack significant transcriptional activity [22]. Therefore, the accurate identification of Oct4A is of paramount importance for studying the function of the Oct4 gene. Wang et al. summarized the following methods for selecting the genuine Oct4A [19]: (1) Since exon 1 is a unique structure of Oct4A, the primers should incorporate exon 1 and span the intron to prevent the amplification of the Oct4 gene series; (2) Given that pseudogenes lack introns and cannot be reverse transcribed into the genome [23], RNAs should be separated using RNase-free DNase I to eliminate DNA contamination. Moreover, when conducting RT-PCR, it is advisable to establish negative control groups with and without reverse transcriptase. (3) Set up specific Oct4A primers. Liedtke et al. recommended two primers to prevent unwanted gene amplification [20]. In certain studies, it has been confirmed that these two primers can be utilized to induce the expression of Oct4A [24, 25]. One of the primers (Oct4_F_P 5'-GATGGCGTACTGTGGGCCC-3'), which carries polymorphisms at the 3' end of the gene, can distinguish Oct4A from pseudogenes located on chromosomes 1 and 8. Nevertheless, caution should be exercised when using primers to differentiate sequences with single nucleotide variance, as Taq DNA polymerase lacks 3' to 5' exonuclease activity, and there is a minute possibility of 3' end mismatch resulting in nucleotide extension [26]. Therefore, when the sample volume of Oct4A is particularly small, pseudogene mismatches can occur in certain circumstances. Another type is the specific mRNA of Oct4A that initiates from the 5' end, which was initially verified as non-existent in all pseudogene groups [20, 27]. Nevertheless, the latest research has disclosed that the mRNA of the pseudogene Oct4p1 also possesses a similar 5' end. The previously mentioned specific mRNA might recognize Oct4p1 instead of Oct4A [19]. Hence, in this situation, it is indispensable to design a set of Oct4p1-specific mRNAs as a control group. (4) The exons of Oct4A contain two specific loci, namely the ApaI and Tsp45I restriction loci. These are absent in other pseudogenes and other Oct4 subgenomes. Based on this, Panagopoulos et al. proposed a PCR/restriction

digestion analysis approach for the specific identification of Oct4A [28]. In the future, more precise methods are needed to identify the Oct4A under study for a better exploration of the function and mechanism of Oct4.

The functional research of Oct4 originated from embryonic cells. It was discovered through studies that Oct4 plays a crucial role in maintaining the self-renewal and multi-potential differentiation of embryonic stem cells [29, 30]. The quantity of Oct4 expression determines the fate of embryonic stem cells. When the expression level of Oct4 declines, embryonic stem cells differentiate into the trophoblast ectoderm lineage. When Oct4 expression is at an intermediate level, embryonic stem cells maintain the stem cell phenotype and continuously self-renew. When the expression level of Oct4 increases, embryonic stem cells can differentiate into the primitive endoderm and mesoderm [30]. Oct4 not only functions in maintaining the self-renewal and differentiation of embryonic stem cells but also participates in regulating the functions of stem cells by forming a complex regulatory network in combination with multiple genes (mainly including SOX2 and Nanog). According to statistics, there are approximately 625 promoter regions of protein-coding genes associated with Oct4 in embryonic stem cells, and approximately 3% of miRNA genes' promoters are related to Oct4, including NANOG, SOX2, FOXO1, CDX2, DPPA4, LEFTY2/EBAF, GJA1/CONNEXIN43, AHAND1, CRIPTO/TDGF1, and ZIC3, among which the majority are common target genes of Oct4, SOX2, and Nanog, totaling 353, such as the quiescent genes ATBF1, DLX1, and ESX1L [29]. Through gene enrichment analysis, it was found that Oct4 is involved in multiple functions and mechanisms, including transcriptional regulation, apoptosis, DNA repair, nucleic acid localization, metabolism, and protein synthesis [31]. Additionally, in early cell reprogramming experiments, researchers successively introduced Yamanaka factors (including Oct4, Sox2, Klf4, and c-Myc) and Thomson factors (including Oct4, Sox2, Nanog, and Lin28) into somatic cells, both of which successfully induced pluripotent stem cells. It can be observed that both groups of factors contain the Oct4 gene [32, 33]. In subsequent studies, Oct4 has been regarded as an important marker for the completion of reprogramming of pluripotent stem cells. The completed pluripotent cells are essentially indistinguishable from embryonic stem cells, further confirming the significance of Oct4 for the pluripotency of stem cells [34]. The latest research has revealed that in human and animal experiments, merely introducing Oct4 can induce pluripotent stem cells, which once again validates the role of Oct4 in reshaping the pluripotency of cells [35, 36]. Therefore, considering the shared characteristics of cancer stem cell and embryonic stem cells, Oct4 has also been incorporated into the research on cancer stem cell. Looijenga et al. first detected various germ cell tumors and somatic cell tumors through immunohistochemistry, confirming that Oct4 is specifically expressed in germ cells and is associated with the multi-potential differentiation of germ cell tumors [37]. Subsequently, researchers have successively discovered high expression of Oct4 cell clusters in lung cancer, digestive tract tumors, head and neck cancer, hematological system tumors, intracranial tumors, and melanoma, all of which possess the characteristics of self-renewal and multi-potential differentiation [8-13]. Similar to the regulation of Oct4 on the fate of embryonic stem cells, when Oct4 is highly expressed, the malignant potential of tumor cells is elevated, accompanied by more primitive and more invasive tumor phenotypes. Conversely, when Oct4 is lowly expressed, the malignancy of tumor cells regresses and they exhibit a better differentiated phenotype [38]. In 2008, Chen et al.'s experiment substantiated this view[39]. The study found that compared with CD133- lung cancer cells, CD133+ lung cancer cells highly express the drug resistance marker ABCG2 and demonstrate resistance to multiple chemotherapy drugs and radiotherapy. When the expression of Oct4 is interfered with by RNA silencing, CD133+ lung cancer cells can differentiate into CD133-lung cancer cells. Knocking out the Oct4 gene can significantly weaken the sphere formation ability and cell invasion ability of CD133+ tumor cells, while promoting the apoptosis of tumor cells. Further research discovered that by transfecting the Oct4 gene into melanoma cells and inoculating them into NOD/SCID mice, the tumorigenic capacity of tumor cells expressing Oct4 is 100 times that of tumor cells not expressing Oct4 [40]. Huang et al. discovered that Oct4 is

highly expressed in liver cancer and adjacent tissues, but not detected in normal liver tissues without cirrhosis, and the difference is statistically significant [16].

Currently, Oct4 has been recommended as a specific marker for cancer stem cell and is utilized for the isolation and identification of cancer stem cell marked by Oct4. Subsequent studies have also revealed that the expression of Oct4 is related to tumor size, type, lymph node metastasis, microvascular metastasis, tumor survival period, drug resistance, and radioresistance, and Oct4 can also be employed to predict the prognosis of tumors [9-13]. Additionally, Hochedlinger et al. identified the oncogenic attribute of Oct4. By overexpressing Oct4 in mice, atypical hyperplasia can occur in epithelial tissues [41]. Ohnishi et al. found that after introducing Yamanaka factors into somatic cells, short-term overexpression of Yamanaka factors (<7 days) can generate teratomas and lead to atypical hyperplasia in other tissue cells. Long-term overexpression of Yamanaka factors in somatic cells may result in the formation of irreversible tumor cells [42]. Simultaneously, they also discovered that the persistent atypical hyperplasia formed after introducing Yamanaka factors can be reversed by inhibiting Oct4 expression with doxycycline. Nishi et al.'s experiments further demonstrated that after introducing Yamanaka factors into non-tumorigenic partially differentiated epithelial cells, cells with malignant tumor phenotypes can be formed, which simultaneously possess the characteristics of cancer stem cell and multiple drug resistances. When transplanted into NOD/SCID mice, they can form xenografts [43]. The fate of cancer stem cell can initially be classified into self-renewal, proliferation, differentiation, apoptosis, and autophagy, etc. Based on the above statements, it can be discerned that Oct4's regulation of the fate of cancer stem cell is multi-faceted and multi-systemic, and the changes in its expression level are closely related to the fate of cancer stem cell.

III. Oct4 And The Self-Renewal Of Cancer Stem Cell

The ability of self-renewal is one of the most significant characteristics of cancer stem cell. Cancer stem cell undergo asymmetric division to generate a daughter tumor stem cell that is identical to the parent cell, while the other daughter cell differentiates into a tumor cell, thereby accomplishing the self-renewal of the parent tumor cell [44]. The distinction between cancer stem cell and normal stem cells lies in the unregulated self-renewal ability of the former, resulting in unrestricted proliferation and the onset, development, and recurrence of tumors. The self-renewal of cancer stem cell is dependent on a microenvironment known as a "niche" [45]. Changes in the niche microenvironment determine the self-renewal, proliferation, or differentiation of cancer stem cell. Under the collective action of a large quantity of extracellular matrix and other factors synthesized and secreted by cells, the cancer stem cell in the niche maintain their inherent characteristics and remain in the G0 phase of the cell cycle for an extended period, in an undifferentiated quiescent state [44]. When the microenvironment is influenced by external factors or disrupted by other development-related genes, the cancer stem cell in the niche will exhibit self-renewal, differentiation, and proliferation [44].

Genes and signaling pathways associated with the self-renewal of cancer stem cell that have been identified to date include Wnt, TGF-β, Notch, Hedgehog, Bmi1, etc. [46]. Additionally, certain miRNAs are also involved in the regulation of the self-renewal of cancer stem cell [47]. Currently, in vitro experiments mainly employ two methods to assess the self-renewal ability of cancer stem cell. One is the colony formation assay, in which methylcellulose or soft agar is utilized to prepare a semi-solid support medium to prevent the migration of tumor cells, thereby forming spatially independent colony clusters [48, 49]. The other is the sphere formation assay, where individual cells are plated at an appropriate dilution concentration to form spatially independent spherical colonies [50]. More importantly, studies have demonstrated that this non-adherent growth without a support is an important characteristic of stem cells [51]. The spherical colonies or clusters obtained

through these two methods can be re-separated and made into single-cell suspensions for new plating, and subsequently, new spherical colonies or clusters are formed again, indicating that cancer stem cell possess unlimited self-renewal ability.

In reality, most current studies merely utilize Oct4 as a marker of the self-renewal of cancer stem cell, and the research objective is to evaluate the regulation of the self-renewal of cancer stem cell by causing changes in the expression level of Oct4 through the modulation of the aforementioned signaling pathways. However, relatively little is known about the signaling pathways or mechanisms through which Oct4 itself regulates the self-renewal of cancer stem cell. Through gene chip technology, it has been discovered that Oct4 primarily participates in the regulation of the self-renewal of embryonic stem cells via the Wnt/β-catenin and TGF-β signaling pathways [29]. Therefore, research on the regulation of the self-renewal of cancer stem cell by Oct4 is largely concentrated on these two signaling pathways, with the majority of studies focusing on cancer stem cell from digestive tract tumors and head and neck tumors [52-54]. Domestic research has revealed that the expression of Oct4 and β-catenin in gastric cancer tissues is significantly higher than that in normal tissues. When the expression of Oct4 is interfered with by silencing RNA, the expression level of β-catenin decreases, suggesting that Oct4 may regulate the self-renewal of tumor cells through the Wnt/β-catenin pathway [52]. Similar phenomena have also been observed in liver cancer. After 24 hours of interference with Oct4 by siRNA, the expression of Oct4 decreased by 70% to 80%, and the expression of β-catenin also declined by approximately 60% [53]. Lee et al. conducted further research on the relationship between Oct4 and Wnt/β-catenin. In head and neck tumor cells, the knockout of β-catenin can markedly reduce the self-renewal ability of cancer stem cell, but overexpression of Oct4 can reverse this situation [54]. Head and neck tumor patients with high levels of both proteins have a significantly poorer prognosis. Chai et al. discovered in their study on liver cancer stem cells that the expression of Oct4 can upregulate the expression level of miRNA-1246, which can subsequently activate the wnt pathway by inhibiting the two degradation complexes of β-catenin (AXIN2 and GSK3β), thereby maintaining the renewal of cancer stem cell [47]. The TGF-β signaling pathway is regarded as having a dual effect on the regulation of cancer stem cell. In liver and gastric cancer tissues, silencing Oct4 leads to a notable increase in TGF-β, while its downstream ELF, Smad3, and Smad4 are significantly decreased [52, 53]. However, in the study of cancer stem cell, it was found that TGF-β can stimulate glioma and liver cancer cells to acquire more characteristics of cancer stem cell, resulting in an increase in the expression of Oct4 [55, 56]. Therefore, the regulatory role of the TGF-β signaling pathway in the self-renewal of cancer stem cell still requires further investigation. Additionally, new research has found that Oct4 can also upregulate the levels of miRNA302a/302b through overexpression, resulting in increased DNA demethylation within cancer stem cell and subsequent activation of self-renewal-related proteins (such as c-IAP1, c-IAP2, and XIAP), ultimately achieving the self-renewal of cancer stem cell [57]. Overall, the regulatory mechanism of Oct4 on the self-renewal of cancer stem cell awaits further research and refinement.

IV. Oct4 And The Pluripotent Differentiation Of Cancer Stem Cell

The pluripotent differentiation is another significant characteristic of cancer stem cell. Precisely due to the pluripotent differentiation of cancer stem cell, tumor heterogeneity emerges. In tumor tissues, the hierarchical expression of cancer stem cell causes tumor cells to differentiate and proliferate in a certain proportion and retains some tumor cells in a quiescent state. When cancer stem cell are stimulated by external factors or disturbed by endogenous secretion factors, it promotes their differentiation process and leads to cell proliferation [58, 59]. Current research has revealed that the pluripotent differentiation of cancer stem cell mainly depends on the environment in which they grow, namely the "niche". Under the combined action of internal and external stimulating factors, they undergo directed differentiation. Existing studies have discovered

that cancer stem cell can differentiate into tumor vascular endothelial cells, different types of tumor cells, and tumor cells with different degrees of invasiveness under the influence of different culture media [60-63].

Currently, there are two methods employed to study the directed differentiation of cancer stem cell: (1) By establishing corresponding culture conditions and adding different types of production factors to achieve directed cell differentiation [64]; (2) By utilizing specific markers or genes on the surface of cancer stem cell and selecting specific types of cells through fluorescence-activated cell sorting (FACS) or magnetic bead sorting for further culture, thereby obtaining specific differentiated cells [65, 66]. Oct4, as a core transcription factor maintaining the pluripotent differentiation potential of stem cells, plays a crucial role in regulating the directed differentiation of cancer stem cell. As mentioned previously, the expression level of Oct4 determines the malignancy of tumor cells and the prognosis of patients [38]. In various tumor cells such as gastric cancer, lung cancer, and liver cancer, a high expression of Oct4 is often accompanied by a low degree of differentiation, increased malignancy, and enhanced tumorigenic ability of tumor cells [9-13]. Chen et al. found that silencing Oct4 in CD133+ lung cancer cells led to their differentiation into CD133- lung cancer cells with lower malignancy [39]. Bunaciu et al. discovered that restricting the expression of Oct4 could induce the activation of the AhR signaling pathway, thereby increasing the differentiation of leukemia cells induced by retinoic acid [67]. In the study of glioma cells, derivatives of tryptophan can inhibit the expression of Oct4 by binding to AhR, resulting in the differentiation of glioma cells, reducing their malignancy and tumorigenic ability [68]. In the study of glioma cells, it was also found that overexpression of Oct4 can increase the expression level of nestin and subsequently reduce the expression of glial fibrillary acidic protein, thereby inhibiting the differentiation of glioma [69]. Lopez-Bertoni et al. found that introducing Oct4 and SOX2 could cause DNA methylation in glioma cells and inhibit the activation of mi148, thereby restoring the stem cell characteristics of glioma cells [70]. More research is still required to understand the mechanism of tumor stem cell differentiation.

V. Oct4 And The Proliferation Of Cancer Stem Cell

The disordered proliferation of tumor cells is the primary pathological feature. Currently, it is believed that cancer stem cell, as the source of tumor initiation, have a greater potential for unlimited proliferation, which is also one of the important characteristics distinguishing normal stem cells from cancer stem cell [71]. Under normal circumstances, cancer stem cell are in a quiescent state, with their cell cycle in the G0 phase. When cancer stem cell are stimulated by external factors or disturbed by endogenous secretory factors, it promotes their differentiation process and leads to cell proliferation [44]. Compared with ordinary tumor cells, cancer stem cell possess stronger proliferation ability, significantly increased tumorigenic and metastatic ability, and concurrently exhibit drug resistance and radiation resistance, resulting in a poor response to radiotherapy and chemotherapy for patients, as well as a shorter survival period and worse prognosis.

Currently, there are four major methods for detecting cell proliferation, including DNA synthesis detection (3H-TdR incorporation method, BrdU method, and EdU method), metabolic activity detection (MTT method, XTT method, MTS method, WST-1 method, CCK-8 method, CFDA-SE method, Alamar Blue and Presto Blue method), cell proliferation-related antigen detection (PCNA and Ki67), and ATP concentration detection [72]. Among them, DNA synthesis detection is the most accurate and reliable method, but it is complex and time-consuming to operate. Therefore, the MTT method or CCK-8 method in metabolic activity detection is currently mainly utilized to evaluate the proliferation ability of tumor cells [72]. Additionally, the tumorigenic ability in vivo can also be employed to assess the proliferation ability of cancer stem cell. Existing studies have demonstrated that Oct4 can promote the proliferation of tumor cells through multiple signaling pathways. Wang et al. found that Oct4 can enhance the proliferation of liver cancer stem cells by activating the PI3K/AKT signaling pathway [73]. Similar results have also been identified in pancreatic cancer, where the knockout of

Oct4 can reduce the expression levels of AKT, PCNA, and MMP2, thereby weakening the proliferation ability of cancer stem cell [74]. Cao et al. discovered in the study of liver cancer stem cells that Oct4 can enhance the proliferation and tumorigenic ability of cancer stem cell by up-regulating the expression of CCND1 and BIRC5 [75]. In the study of esophageal cancer, it was found that the knockout of Oct4 can reduce the expression level of survivin in cancer stem cell, induce apoptosis of cancer stem cell, and cause cell differentiation to be arrested at the G2 phase, resulting in a decrease in the proliferation ability of esophageal cancer cells [76]. Jen et al. found that Oct4 can up-regulate the expression levels of NEAT1 and MALAT1, enhancing the proliferation ability of lung cancer cells [77].

VI. Oct4 And The Apoptosis Of Cancer Stem Cell

Cell apoptosis is defined as a physiological process in which the organism undergoes active death in accordance with its own program [78]. cancer stem cell, as immortal cells, are not only related to their self-renewal and proliferation but also to the disorder in the cell apoptosis process. Compared to highly differentiated tumor cells, cancer stem cell can better resist apoptosis induced by various factors [79]. The apoptosis of cancer stem cell can mainly be triggered through pathways such as the death receptor-mediated pathway, the mitochondrial-cytochrome C pathway, the endoplasmic reticulum pathway, and the AIF-mediated non-caspase-dependent pathway [79, 80].

Currently, commonly used methods for detecting cell apoptosis include caspase activity detection, DNA fragmentation analysis and morphological analysis, Annexin V staining, membrane potential and other mitochondrial detection experiments, and flow cytometry detection methods [81, 82]. Previous studies have confirmed that as an anti-apoptotic gene, Oct4 can inhibit the apoptosis of cancer stem cell through multiple pathways [39, 76, 83-85]. Chen et al. used siRNA to knock out Oct4 in CD133+ lung cancer stem cells, which could induce the activation of Caspase 3/PARR, promote the apoptosis of cancer stem cell, and significantly reduce their sphere formation ability and proliferation ability [39]. In the study of pancreatic cancer, it was found that after transfection of Oct4 siRNA into PACN1 cells, the mitochondrial membrane potential of the cells decreased, PARP was cleaved, Caspase 3 was activated, and tumor cell apoptosis was induced [83]. In the study of esophageal cancer, inhibition of Oct4 expression could also increase the expression level of Survivin, thereby inducing the apoptosis of cancer stem cell [76]. In colon cancer stem cells, Wen et al. obtained similar results. After knocking out Oct4, the expression level of Survivin increased, and then the apoptosis of cancer stem cell was increased by activating the STAT3 signaling pathway [84]. Hu et al. found that inhibition of Oct4 expression by shRNA could reduce the expression level of Tcl1 and down-regulate the level of p-Ser.473-Akt1, thereby inducing the apoptosis of cancer stem cell [85]. Wang et al. found that overexpression of Oct4 could increase the expression of miRNA-125b, and then inhibit the expression of BAK protein, ultimately blocking the apoptosis of cervical cancer cells [86].

VII. Oct4 And The Autophagy Of Cancer Stem Cell

Cell autophagy is a lysosome-dependent degradation pathway widely existing in eukaryotic cells. Broadly speaking, it can be roughly divided into three types: macroautophagy, microautophagy, and chaperone-mediated autophagy [87, 88]. Autophagy plays a dual role in the occurrence and development of tumors. On one hand, autophagy can degrade damaged proteins and organelles within cells, generating new amino acids, fatty acids, and other degradation products to provide energy for cell growth and proliferation [89]. On the other hand, autophagy itself is also a death mechanism, which can kill cells by degrading cellular components (such as catalytic enzymes, mitochondria, etc.) or by non-selectively degrading cellular contents until the cells cannot survive [90-91].

Currently, the main methods for detecting cell autophagy include in vitro static detection (electron microscopy detection, acidic autophagic vesicles, autolysosomes detection, LC3 detection), in vitro dynamic detection (electron microscopy LC3 flux detection, protein degradation), and in vivo detection (in vivo detection through fluorescence microscopy) [92]. In the study of cancer stem cell, it was found that autophagy can control the internal environment of cancer stem cell and enhance their self-renewal and proliferation abilities [93]. In the research on osteosarcoma, it was found that the decline in autophagic capacity can reduce the stemness of cancer stem cell and increase their sensitivity to chemotherapy drugs [94]. In the study of cervical cancer, it was found that the expression levels of autophagy-related proteins Beclin 1 and LC3B in cancer stem cell in the spheroid state were significantly increased, and the apoptosis wave induced by starvation was also significantly increased [95]. However, in some other studies, it was found that when drugs induce autophagy in cancer stem cell, the autophagic wave can lead to the loss of stemness and apoptosis of cancer stem cell [96-97]. In the experiment by Jiang et al., it was found that the oncolytic adenovirus Delta-24-RGD activates autophagy in brain cancer stem cell by acting on the abnormal p16INK4/Rb pathway, leading to the death of tumor cells [98]. Overall, autophagy in cancer stem cell provides energy for maintaining their stemness by digesting senescent organelles, but when the external microenvironment changes, excessive activation of autophagy can induce apoptosis and necrosis of cancer stem cell.

Currently, there are very few studies on the mechanism by which Oct4 regulates autophagy in cancer stem cell. Most studies only use Oct4 as a characteristic of cancer stem cell to measure the regulation of autophagy on cancer stem cell. In the study by Sharif et al., it was found that knocking out Oct4 led to a decrease in the expression levels of autophagy-related proteins ATG5, ATG7, ATG12, LC3A-II, and LC3BII, a downregulation of the mRNA levels of ATG5, ATG7, ATG12, BECN1, and ULK1, and a reduction in autophagic vacuoles. At the same time, Oct4-knocked-out cancer stem cell expressed lower levels of SQSTM1/SQSTM1 mRNA and protein, resulting in a decrease in the level of autophagic degradation and ultimately a decline in the pluripotent differentiation ability of cancer stem cell [93]. Additionally, the Oct4 protein is degraded through the molecular chaperone-mediated autophagy pathway under long-term starvation conditions, thereby promoting the differentiation of nasopharyngeal carcinoma stem cells [99]. In a somatic cell reprogramming experiment, it was found that the transfection of Oct4 can inhibit autophagy-related genes [100]. Therefore, the regulation of autophagy by Oct4 in cancer stem cell still requires further investigation.

VIII. Summary

The research on cancer stem cell plays a crucial role in the origin, metastasis, recurrence, and drug resistance of tumors. How to systematically eliminate cancer stem cell is of significant importance for improving the prognosis of tumor patients. Oct4, as a core transcription factor that comprehensively regulates the self-renewal, proliferation, differentiation, and many other fates of cancer stem cell, in-depth research on it will provide a glimmer of hope for the complete treatment of tumors.

References

[1] Chen W,Zheng R,Baade PD, Et Al.Cancer Statistics In China, 2015.CA Cancer J Clin, 2016, 66(2):115-132.

[2] Reya T,Morrison SJ,Clarke MF,Et Al.Stem Cells, Cancer, And Cancer Stem Cells.Nature,2001,414(6859):105-111.

[3] Patrawala L, Calhoun T, Schneider-Broussard R, Et Al. Side Population Is Enriched In Tumorigenic, Stem-Like Cancer Cells, Whereas ABCG2+ And ABCG2-Cancer Cells Are Similarly Tumorigenic. Cancer Res, 2005, 65(14): 6207-6219.

[4] Bonnet D, Dick JE.Human Acute Myeloid Is Organized As A Hierarchy That Originates From A Primitive Hematopoietic Cell.Nat Med, 1997,3(7):730-737.

[5] Al-Hajj M, Wicha Ms, Benito-Hemadez A.Prospective Identification Of Tumorigenic Breast Cancer Cells.Proc Natl Acad Sci

USA,2003,100(7):3983-3988

[6] Singh SK, Clarke ID, Terasaki M, Et Al.Identification Of A Cancer Stem Cell In Human Brain Tumors. Cancer Res, 2003 ,63(18):5821-5828.

[7] Eramo A, Lotti F, Sette G, Et Al.Identification And Expansion Of The Tumorigenic Lung Cancer Stem Cell Population.Cell Death Differ,2008,15(3):504-514.

[8] Yuan F. Expression Of Oct4 In HCC And Modulation To Wnt /B -Catenin And TGF-B Signalpathways . Mol Cell Biochem,2010,343 : 155 -162

[9] Ong C W,Kim L G. CD133 Expression Predicts For Non-Response To Chemotherapy In Colorectal Cancer. Mod Pathol, 2010,23(3) : 450 -457

[10] Ponti D, Costa A . Isolation And In Vitro Propagation Of Tumorigenic Breast Cancer Cells With Stem /Progenitor Cell Properties. Cancer Res, 2005, 65(13) : 5506 - 5511

[11] Houghton J, Stoicov C, Nomura S, Et Al. Gastric Cancer Originating From Bone Marrow-Derived Cells. Science, 2004,306(5701):1568-1571.

[12] Rappa G,Fodstad O,Lorico A.The Stem Cell-Associated Antigen CD133 (Prominin-1) Is A Molecular Therapeutic Target For Metastatic Melanoma. Stem Cells, 2008,26(12),3008-17

[13] Zhang Y, Peng J, Zhang H, Et Al.Notch1 Signaling Is Activated In Cells Expressing Embryonic Stem Cell Proteins In Human Primary Nasopharyngeal Carcinoma. J Otolaryngol Head Neck Surg, 2010,39(2) : 157 -166.

[14] Goodell MA, Brose K, Paradis G, Et Al. Isolation And Functional Properties Of Murine Stem Cells That Are Replicating In Vivo. J Exp Med, 1996, 183(4):1797-1806.

[15] Ho MM, Ng AV, Lam S, Et Al. Side Population In Human Lung Cancer Cell Lines And Tumors Is Enriched With Stem-Like Cancer Cells. Cancer Res, 2007, 67(10): 4827-4833.

[16] Huang PZ, Lu CL, Li BK, Et Al. OCT4 Expression In Hepatocellular Carcinoma And Its Clinical Significance. Chin J Cancer, 2010, 29 (1):111-116.

[17] Ge N, Lin HX, Xiao XS, Et Al. Prognostic Significance Of Oct4 And Sox2 Expression In Hypopharyngeal Squamous Cell Carcinoma. J Transl Med, 2010, 8: 94.

[18] 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 (44): 33554-33565

[19] Wang X , Dai J.Concise Review: Isoforms Of OCT4 Contribute To The Confusing Diversity In Stem Cell Biology.Stem Cells, 2010 ,28(5):885-93.

[20] Liedtke S, Enczmann J, Waclawczyk S Et Al. Oct4 And Its Pseudogenes Confuse Stem Cell Research. Cell Stem Cell, 2007,1(4):364–366

[21] Pain D, Chirn GW, Strassel C Et Al. Multiple Retropseudogenes From Pluripotent Cell-Specific Gene Expression Indicates A Potential Signature For Novel Gene Identification. J Biol Chem, 2005,280(8):6265–6268.

[22] Zhao S, Yuan Q, Hao H, Et Al. Expression Of OCT4 Pseudogenes In Human Tumours: Lessons From Glioma And Breast Carcinoma . J Pathol, 2011, 223 (5): 672-682.

[23] Harrison PM, Zheng D, Zhang Z, Et Al. Transcribed Processed Pseudogenes In The Human Genome: An Intermediate Form Of Expressed Retrosequence Lacking Protein-Coding Ability. Nucleic Acids Res, 2005,33(8):2374–2383

[24] Seo KW, Lee SR, Bhandari DR Et Al. OCT4A Contributes To The Stemness And Multi-Potency Of Human Umbilical Cord Blood-Derived Multipotent Stem Cells (Hucb-Mscs). Biochem Biophys Res Commun, 2009, 384(1):120–125.

[25] Sotomayor P, Godoy A, Smith GJ Et Al. Oct4A Is Expressed By A Subpopulation Of Prostate Neuroendocrine Cells. Prostate, 2009,69(4): 401–410.

[26] Gale JM, Tafoya GB. Evaluation Of 15 Polymerases And Phosphorothioate Primer Modification For Detection Of UV-Induced C:G To T:A Mutations By Allele-Specific PCR. Photochem Photobiol, 2004, 79(5): 461–469.

[27] Wang X, Zhao Y, Xiao Z Et Al. Alternative Translation Of OCT4 By An Internal Ribosome Entry Site And Its Novel Function In

Stress Response. Stem Cells, 2009, 27(6):1265–1275.

[28] Panagopoulos I, Moller E, Isaksson M Et Al. A PCR/Restriction Digestion Assay For The Detection Of The Transcript Variants 1 And 2 Of POU5F1. Genes Chromosomes Cancer, 2008, 47(6):521–529.

[29] Boyer LA, Lee TI, Cole MF Et Al. Core Transcriptional Regulatory Circuitry In Human Embryonic Stem Cells. Cell, 2005,122(6):947–956.

[30] Niwa H, Miyazaki J, Smith AG. Quantitative Expression Of Oct-3/4 Defines Differentiation, Dedifferentiation Or Self-Renewal Of ES Cells. Nat Genet, 2000 ,24(4):372–376.

[31] Campbell PA 1, Perez-Iratxeta C, Andrade-Navarro MA,Et Al.Oct4 Targets Regulatory Nodes To Modulate Stem Cell Function.Plos One, 2007,2(6):E553.

[32] Takahashi K, Yamanaka S. Induction Of Pluripotent Stem Cells From Mouse Embryonic And Adult Fbroblast Cultures By Defned Factors. Cell, 2006, 126(4):663-676

[33] Yu J, Vodyanik MA, Smuga-Otto K, Et Al. Induced Pluripotent Stem Cell Lines Derived From Human Somatic Cells. Science, 2007,318(5858):1917-1920.

[34] Wernig M, Meissner A, Foreman R, Et Al. In Vitro Reprogramming Of Fbroblasts Into A Pluripotent ES-Cell-Like State. Nature, 2007, 448(7151):318-324.

[35] Kim JB, Sebastiano V, Wu G, Et Al. Oct4-Induced Pluripotency In Adult Neural Stem Cells. Cell, 2009, 136(3):411-419.

[36] Kim JB, Greber B, Arauzo-Bravo MJ, Et Al.Direct Reprogramming Of Human Neural Stem Cells By OCT4. Nature, 2009,461(7264):649-653.

[37] Looijenga LH, Stoop H, De Leeuw HP, Et Al. POU5F1 (OCT3 /4) Identifies Cells With Pluripotent Potential In Human Germ Cell Tumors. Cancer Res, 2003, 63(9) : 2244 -2250.

[38] Gidekel S, Pizov G, Bergman Y, Et Al. Oct-3/4 Is A Dose-Dependent Oncogenic Fate Determinant. Cancer Cell, 2003,4(5):361–70.

[39] Levings PP 1, Mcgarry SV, Currie TP,Et Al.Expression Of An Exogenous Human Oct-4 Promoter Identifies Tumor-Initiating Cells In Osteosarcoma. Cancer Res, 2009,69(14):5648-55.