OCT-4, an Embryonic Stem Cell Marker Expressed in Breast, Brain and Thyroid Carcinomas Compared to Testicular Carcinoma

Madjd Z [1,2,3,4], Hashemi F[1,3], Shayanfar N[1], Farahani E [3], Zarnani AH[5,6], Sharifi AM[2,7], Akbari ME[4]

Abstract

Introduction: Cancer stem cells are a small subpopulation of cells within a tumor which are responsible for maintaining the tumor mass. A number of factors such as OCT-4 that govern the fate of adult stem cells also play a role in malignant cell transformation. OCT-4 is a key regulator of self-renewal in embryonic stem cells; its expression is potentially correlated with tumorigenesis and can affect some aspects of tumor behavior such as tumor recurrence or resistance to therapies.

Methods: We have investigated the potential expression of OCT-4 on a panel of tumors including breast, brain, thyroid and testicular carcinomas, using immunohistochemistry. The level of expression of OCT-4 was then compared to different tumor types and degree of differentiation.

Results: OCT-4 was expressed at the highest levels on nuclear site of seminoma compared with other tumors. The expression of OCT-4 was detectable in both nucleus and the cytoplasm of almost all breast tumors, but it was detectable at much lower level in normal breast tissues. OCT-4 expression was noted on poorly differentiated papillary carcinoma of thyroid compared to normal follicles of thyroid gland adjacent to the tumor.

Conclusion: Breast carcinomas and papillary carcinomas of thyroid express elevated levels of embryonic stem cell gene OCT-4, suggesting that these tumors may contain cells indicative of embryonic-like stem cells. Identification of cancer stem cells in different malignant tumors may be useful for prognostic evaluation and administration of a new treatment which target this sub-population of tumor cells.

Keywords: cancer stem cells, breast cancer, seminoma, oct-4

1. Dep Pathology, Iran University of Medical Sciences, Tehran, Iran
2. Cellular Molecular Research Centre, Iran University of Medical Sciences, Tehran, Iran
3. Oncopathology Research Centre, Iran University of Medical Sciences, Tehran, Iran
4. Cancer Research Centre, Shahid Beheshti University of Medical Sciences, Tehran, Iran
5. Nanotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
6. Immunology Research Center, Iran University of Medical Sciences, Tehran, Iran
7. Dep of Pharmacology, Iran University of Medical Sciences, Tehran, Iran

Corresponding Author:
Zahra Madjd, MD PhD
Assistant professor of Immunopathology
Tel :(#98)2182943212
Email: zahra.madjd@yahoo.com

Received: 19 Oct. 2009
Accepted: 24 Nov. 2009
IJCP 2009; 4: 167-173

Introduction

Cancer stem cells (CSC) are a subpopulation of tumor cells having the capacity to self-renew and generate tumor heterogeneity. According to the cancer stem cell concept, CSCs have high renewal capacity and can be generated through dysregulation of self renewal process in normal stem cells [1-4].

On the other hand, cancer stem cells are responsible for maintaining the tumor mass as they are capable to self- renew to generate further CSCs as well as non-tumorigenic cells. These facts can be considered a major advance in cancer research [5].

OCT-4 is a key transcription factor that is compulsory for the self-renewal and pluripotency characteristics of embryonic stem (ES) cells and germ cells. Expression of OCT-4 is limited to pluripotent cells; and the level of expression decreases with the commencement of differentiation and loss of pluripotency in these cells [6, 7]. OCT-4 is largely expressed in human germ cell tumors [8] and its expression has also been identified in several somatic cancers including prostate, bladder and breast carcinomas [9, 10].

It has been anticipated that OCT-4 acts as a multifunctional factor in cancer and stem cell biology. OCT-4 enhances the malignant potential of embryonic stem cells in a dose dependent way [11].

Expression of OCT-4 in epithelial tissues causes dysplasia by inhibiting cellular differentiation in a way similar to that in the ES cells [12, 13]. Immunohistochemical detection of OCT-4 is highly sensitive and specific for the diagnosis of seminoma and embryonal carcinoma metastatic from the testis. Establishing germ cell origin for metastatic tumors has important implications for assessing patient prognosis and treatment options [14].

There are similarities between embryonic tissues and cancer with respect to their substantial capacity for proliferation and differentiation. These observations led to the hypothesis that resting embryonic stem cells may exist in adult tissues and that upon activation these cells may obtain the ability to cause cancer [15-17].

Although the expression of embryonic stem cell marker OCT-4 in germ cell tumors has been broadly studied, its expression in many somatic cancers is still unknown. The aim of the present study was to explore the potential expression of stem cell marker OCT-4 in a panel of tumors including breast, brain and thyroid carcinomas, and to evaluate its correlation with tumor types and degree of differentiation.

Materials and Methods

Patients and tumour samples

In this study, Paraffin-embedded tumor tissues were used from patients with breast, thyroid, brain carcinomas as well as seminoma who underwent surgery or biopsy at Rasool akram and Firoozgar Hospitals (two major public referral centers in Tehran). Patients' characteristics (age) and tumor characteristics including histological grade, vascular invasion, tumor size, lymph node metastasis, and tumor type were collected and recorded in a database. This research was approved by the IUMS Research Ethics Committee. Patients' and tumors' characteristics are summarized in Table 1-3.

Immunohistochemistry

Four- micrometer-thick sections of appropriately selected, formalin-fixed, paraffin-embedded surgical pathology specimens were used for immunohistochemical staining as described previously [18, 19]. After deparaffinization, sections were immersed in methanol containing 0.3% hydrogen peroxide for 15 minutes to block endogenous peroxidase activity. Nonspecific binding sites were blocked appropriately using Protein Block (Dako, Denmark) for 20 minutes. Antigen retrieval was performed by heating sections in citrate buffer (pH: 6.0) for 10 minutes using autoclave.

OCT-4 immunostaining was accomplished with a mouse monoclonal anti-human OCT-4 antibody (ab59545, abcam, Cambridge, UK; at 1:5 dilution) for 1 hour at room temperature (RT) using Labeled StreptAvidin Biotin (LSAB) technique (Dako, Denmark).

The protocol was followed with the addition of 3, 3'-diaminobenzidine (DAB, Dako) to achieve visualization of the antigen. In the final step, sections were lightly counterstained with haematoxylin (Dako), dehydrated in alcohol, cleared in xylene (Dako) and mounted for examination.

The primary antibody being omitted from the negative control and seminoma sections were applied as positive controls for both antibodies.

Evaluation of immunostaining

The tissue sections were initially evaluated using a semi-quantitative system in a coded manner by one author (ZM) who had no knowledge of the clinical and pathological parameters. The results obtained were confirmed by the second observer (NS) using a multi-headed microscope; and the two observers reached a consensus. Semi-quantitative scoring systems are the historical standard for the assessment of immunostaining of tissue sections. These systems usually rely on the subjective assessment of multiple independent observers blinded to the patient outcomes and the clinicopathological data [18, 20, 21].

The percentages of cells that stained positively for OCT-4 were estimated, and the staining intensity was classified as negative (0), weak (1), moderate (2), or strong (3) as described previously [22-25].

Initially, the slides were scanned at 10x magnification to obtain a general impression of the overall distribution of the tumor cells, and the positive cells were then assessed semi quantitatively at higher magnifications and the final scores were given.

Results

Demographics of patients and tumour characteristics

The mean age of the 18 breast cancer patients was 51 years at the time of the diagnosis (26-86). The most common histological type was invasive ductal carcinoma, comprising 88% of the cases. The majority of tumors were grade 3, and only 22% of the cases were grade 1. Thirty seven percent of the tumors were 3 cm or less in size. Of the patients with known lymph node status, 23% had one or two auxiliary nodes involved. Vascular invasion was observed in 33% of the tumors.

The mean age of thyroid carcinoma patients was 47 years (21-80). The majority of the tumors were papillary carcinoma (70%), whereas the rest of the tumors were follicular carcinoma (23%) or medullary carcinoma (7%). Lymph node was involved in 30% of the thyroid carcinoma patients. The size of the tumors was 3 cm or less in 83% of these cases.

Brain tumors comprised of meningioma (48%), and glioblastoma (24%); the rest of the tumors were metastatic carcinomas, schwannoma and ologodendroglioma. The majority of these tumors were grade 1 (54%). Forty percent of the brain tumors were 3 cm or less in size.

Expression of OCT-4 in malignant and nonmalignant tissues

Using the monoclonal anti-OCT-4 antibody, we examined the tissue distribution and sub cellular localization of OCT-4 on a panel of tumor tissues by immunohistochemistry (IHC). We used paraffin sections of seminoma as a positive control, which was known to have a nuclear localization for OCT-4.

Table 1. *Patients and Breast tumours characteristics*

Vascular Invasion	Tumour Size (cm)	LN Metastasis	Grade	Age	Tumour Type	
Neg	2.5	Pos	2	26	In situ & Invasive Ductal Carcinoma	1
Pos	NA	NA	3	40	Invasive Ductal Carcinoma	2
NA	NA	NA	3	86	Infiltrative Ductal Carcinoma	3
Pos	5	NA	2	49	In situ & Invasive Ductal Carcinoma	4
NA	2.5	NA	1	49	Invasive Ductal Carcinoma	5
NA	NA	Pos	NA	53	Invasive Ductal Carcinoma	6
Neg	2	NA	1	57	Invasive Ductal Carcinoma, (NOS type)	7
NA	NA	NA	3	47	Invasive Ductal Carcinoma, (Recurrence Tumour)	8
NA	7	Pos	3	46	Invasive Ductal Carcinoma	9
Pos	6	Pos	3	72	Invasive Ductal Carcinoma, (NOS type)	10
NA	7	NA	2	33	In situ & Invasive Ductal Carcinoma	11
Pos	3.2	Neg	1	61	Invasive Ductal Carcinoma, (cribriform &Papillary)	12
NA	13	Neg	3	73	Sarcomatoid Carcinoma (Pleomorphic Carcinoma)	13
Pos	3	NA	1	41	Invasive Ductal Carcinoma (NOS type)	14
Pos	5	NA	NA	41	Invasive Ductal Carcinoma	15

NA: None Applicable

Table 2. *Patients and thyroid tumours characteristics*

Vascular Invasion	Tumour Size (cm)	LN Metast	degree of differentiation	Age	Tumour Type	
NA	0.9	NA	NA	55	Papillary Carcinoma,	1
Pos	2	Neg	NA , Multi- centric	53	Medullary Carcinoma	2
Neg	6	Neg	NA	67	Follicular Carcinoma ,(Hurthle cell variant)	3
NA	NA	Pos	Recurrence	60	Papillary Carcinoma	4
Neg	2.2	Neg	Well-differentiated	25	Papillary Carcinoma, Classic Type	5
Neg	0.5	Neg	Well-differentiated	53	Papillary Carcinoma, Classic Type	6
NA	NA	NA	Minimally invasive	31	Follicular Carcinoma	7
NA	0.7	Neg	NA	53	Papillary Carcinoma ,Follicular variant	8
NA	3	Pos	Well-differentiated	35	Papillary Carcinoma	9
Neg	1.8	NA	Anaplastic (undifferentiated)	80	Papillary Carcinoma,	10
Neg	2	Pos	NA	30	Papillary Carcinoma, Classic Type	11
Neg	0.1	Pos	NA	30	Papillary Carcinoma, Classic Type	12
Neg	2.5	NA	Well-differentiated	72	Papillary Carcinoma	13
Neg	5	NA	NA , No Multi- centric	21	Papillary Carcinoma Follicular variant	14

Table 3. *Patients and brain tumors characteristics*

Vascular Invasion	Tumor Size(cm)	LN Metast	Grade	Tumor Type	
NA	3.5	NA	1	Meningioma, psammomatous type	1
NA	4.5	NA	NA	Metastatic Ductal Breast Carcinoma	2
NA	7	NA	1	Transitional Meningioma	3
NA	7	NA	NA	Oligodendroglioma, recurrence	4
NA	NA	NA	2	Hemangiopericytoma	5
NA	7	NA	1	Meningioma, syncytial type	6
NA	3.5	NA	1	Meningotheliomatous meningioma	7
NA	2	NA	1	Transitional Meningioma	8
NA	3	NA	4	Glioblastoma multiforme	9
NA	2	NA	6	Glioblastoma multiforme Recurrent tumor	10

In this study, OCT-4 was consistently expressed at the highest levels on nuclear site of seminoma compared with all other tumors [Fig1A]. Therefore, seminoma was used as a positive control; and the level of expression of these proteins in other tumors was compared with seminoma. No immunoreactivity was observed in negative controls, which were incubated in the absence of primary antibody.

Tissue distribution of OCT-4 protein in breast, thyroid and brain tumors

Breast tumours

OCT-4 was detectable in both nucleus and the cytoplasm of breast carcinoma cells, whereas normal breast ducts did not express detectable levels of these proteins [Fig1B]. We clearly observed OCT-4 protein expression in clusters of cells; particularly in regions close to the adipocytes. The intensity of staining was variable in positive cells. OCT-4 was significantly over expressed in poorly differentiated breast carcinoma compared to well differentiated tumours of the patients.

Thyroid tumours

Expression of OCT-4 was also noted on poorly differentiated papillary carcinoma of thyroid compared to normal follicles of thyroid gland adjacent to tumor. Interestingly, the level of expression of OCT-4 on follicular and medullary carcinoma was much lower than papillary carcinomas [Fig1C, 1D].

Brain tumours

Over expression of OCT-4 was found in glioblastoma multiform (grade IV), oligodendrogelioma and transitional meningioma compared to normal adjacent tissues [Fig1E]. OCT-4 was significantly over expressed in metastatic brain tumors rather than other brain tumor types [Fig1F]. The level of expression of OCT-4 protein in high grade tumors was much higher than lower grade tumors.

Discussion

A key goal in cancer research is to identify the mechanism by which cancer stem cells arise and self-renew themselves. It is suggested that the activation of an embryonic stem cell-like transcriptional program in differentiated adult cells may lead to pathological self-renewal characteristics of cancer stem cells [13].

Cancer Stem Cells (CSC) arises from their normal stem cell counterparts that undergo accumulation of genetic changes until the cells acquire a malignant phenotype. They have qualities significant of normal tissue stem cells including self-renewal, prolonged survival, and the ability to give rise to cells with more differentiated characteristics. Many studies have been performed to identify cancer stem cells in various malignancies, and to define the cells of origin. According to this role for cancer stem cells in tumorigenesis and accepting that these cells are generated through uncontrolled self renewal of normal stem or progenitor cells, it is crucial to study the expression and involvement of stem cell's self-renewal regulator genes in carcinogenesis

In the present study, we examined the expression of a well-known self-renewal regulatory factor, OCT-4 protein, in formalin fixed paraffin embedded (FFPE) sections of a panel of somatic cancers employing immunohistochemistry. We observed nucleolus and some cytoplasmic distribution of OCT- 4 in most of the tumors with no or weak immunoreactivity in normal cells adjacent to the tumors. Our results confirmed the same sub cellular localization for OCT-4 in other studies [9, 13, 26].

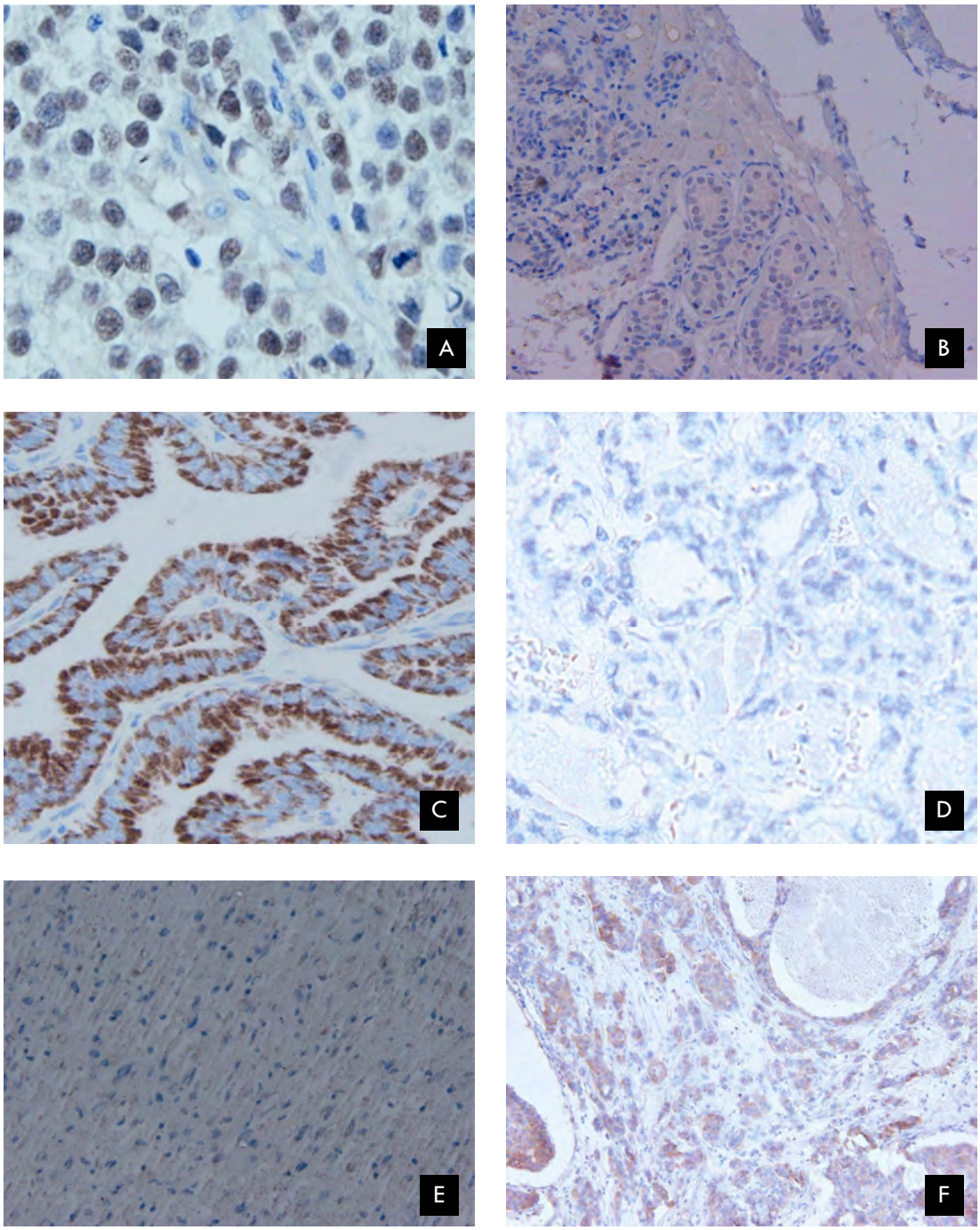

Figure1. *Immunohistochemistry of OCT-4 protein expression in paraffin embedded tissue.*

A) *Strong nuclear staining of seminoma as positive control (magnification X40).* ***B)*** *Nuclear staining of OCT-4 in breast carcinoma with no expression of OCT-4 in normal duct of breast .* ***C)*** *Expression of OCT-4 in papillary carcinoma of thyroid.* ***D)*** *No expression of OCT-4 protein in follicular carcinoma of thyroid* ***E)*** *OCT-4 expression in brain carcinoma and* ***F)*** *Brain metastatic tumour*

We observed the highest expression of OCT-4 on nuclear site of seminoma compared with all other tumors in this study. Several other studies have addressed the expression of OCT-4 in seminoma and carcinoma in situ [26-28]. Therefore, we used seminoma tissues as positive control to compare to the level of expression of OCT-4 in other tumors.

Expression of OCT-4 was observed in both nucleus and the cytoplasm of breast carcinoma cells with a variable intensity among positive cells, suggesting that the cells within the tumors are heterogeneous in term of these proteins. Similar observation has been reported previously by Ezeh et al, who detected stem cell marker OCT4 in a few number of cases using immunohistochemistry and PCR (polymerase chain reaction) [26]. Poorly differentiated breast carcinomas significantly over expressed OCT-4 compared to well differentiated tumors of the patients and this was in co ordinance with Schoenhals study [13].

Our results also showed an over expression of OCT-4 on poorly differentiated papillary carcinoma of thyroid compared to normal follicles of thyroid gland adjacent to tumor. Interestingly, follicular and medulary carcinomas showed much lower expression than papillary carcinomas. OCT-4 expression has been previously reported only in one case of poorly differentiated carcinoma of thyroid [29].

In addition, we found an over expression of OCT-4 protein in oligodendroglioma, glioblastoma multiforme (grade IV), transitional meningioma, and also in metastatic brain tumors. This was in line with a review study using publicity available gene expression data that also emphasized the over expression of OCT-4 in oligodendroglioma, glioblastoma multiforme (IV) and astrocytoma [13]. OCT-4 protein was over expressed in high grade brain tumors (glioblastoma grade IV) compared to lower grade tumors as previously reported [13]. In summary, the present study has shown that pluripotency factor OCT-4 is over expressed in most of cancer types investigated including breast, thyroid and brain carcinoma. The expression of this stem cell marker was also associated with tumor progression or poor prognosis. These findings propose that stem cell genes such as OCT-4 can play a direct role in different types of carcinoma progression or can act as a marker of tumorigenesis. Further studies are needed to clarify the role of these pluripotent transcription factors in these cancers.

Acknowledgements

This study was supported by a grant from the Cellular Molecular Research Centre, Iran University of Medical Sciences (grant number 159).

Conflict of interests

The authors declare that they have no conflict of interests.

References

1. Al-Hajj M, Clarke MF. Self-renewal and solid tumor stem cells. Oncogene. 2004 Sep 20; 23(43):7274-82.

2. Chang CC. Recent translational research: stem cells as the roots of breast cancer. Breast Cancer Res. 2006; 8(1):103.

3. Pardal R, Clarke MF, Morrison SJ. Applying the principles of stem-cell biology to cancer. Nat Rev Cancer. 2003 Dec; 3(12):895-902.

4. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature. 2001 Nov; 414(6859):105-11.

5. Romano G. The role of adult stem cells in carcinogenesis. Drug News Perspect. 2005 Nov; 18(9): 555-9.

6. Matin MM, Walsh JR, Gokhale PJ, Draper JS, Bahrami AR, Morton I, et al. Specific knockdown of Oct4 and beta2-microglobulin expression by RNA interference in human embryonic stem cells and embryonic carcinoma cells. Stem Cells. 2004; 22(5):659-68.

7. Niwa H, Miyazaki J, Smith AG. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nat Genet. 2000 Apr; 24(4):372-6.

8. Clark AT, Rodriguez RT, Bodnar MS, Abeyta MJ, Cedars MI, Turek PJ, et al. Human STELLAR, NANOG, and GDF3 genes are expressed in pluripotent cells and map to chromosome 12p13, a hotspot for teratocarcinoma. Stem Cells. 2004; 22(2):169-79.

9. Atlasi Y, Mowla SJ, Ziaee SA, Bahrami AR. OCT-4, an embryonic stem cell marker, is highly expressed in bladder cancer. Int J Cancer. 2007 Apr 1; 120(7):1598-602.

10. Sotomayor P, Godoy A, Smith GJ, Huss WJ. Oct4A is expressed by a subpopulation of prostate neuroendocrine cells. Prostate. 2009 Mar 1; 69(4):401-10.

11. Gidekel S, Pizov G, Bergman Y, Pikarsky E. Oct-3/4 is a dose-dependent oncogenic fate determinant. Cancer Cell. 2003 Nov; 4(5):361-70.

12. Hochedlinger K, Yamada Y, Beard C, Jaenisch R. Ectopic expression of Oct-4 blocks progenitor-cell differentiation and causes dysplasia in epithelial tissues. Cell. 2005 May 6; 121(3):465-77.

13. Schoenhals M, Kassambara A, De Vos J, Hose D, Moreaux J, Klein B. Embryonic stem cell markers expression in cancers. Biochem Biophys Res Commun. 2009 May 29; 383(2):157-62.

14. Cheng L. Establishing a germ cell origin for metastatic tumors using OCT4 immunohistochemistry. Cancer. 2004 Nov 1; 101(9):2006-10.

15. Sell S. Stem cell origin of cancer and differentiation therapy. Crit Rev Oncol Hematol. 2004 Jul; 51(1):1-28.

16. Cohnheim J. Ueber entzundung und eiterung. Path Anat Physiol Klin Med. 1867; 40:1-79.

17. Durante F. Nesso fisio-pathologico tra la struttura dei nei materni e la genesi di alcuni tumori maligni. Arch Memori ed Osservazioni di Chirugia Practica 1874; 11:217-26.

18. Madjd Z, Mehrjerdi AZ, Sharifi AM, Molanaei S, Shahzadi SZ, Asadi-Lari M. CD44+ cancer cells express higher levels of the anti-apoptotic protein Bcl-2 in breast tumours. Cancer Immun. 2009; 9:4.

19. Madjd Z, Pinder SE, Paish C, Ellis IO, Carmichael J, Durrant LG. Loss of CD59 expression in breast tumours correlates with poor survival. J Pathol. 2003 Aug; 200(5):633-9.

20. Abd El-Rehim DM, Pinder SE, Paish CE, Bell J, Blamey RW, Robertson JF, et al. Expression of luminal and basal cytokeratins in human breast carcinoma. J Pathol. 2004 Jun; 203(2):661-71.

21. Putti TC, El-Rehim DM, Rakha EA, Paish CE, Lee AH, Pinder SE, et al. Estrogen receptor-negative breast carcinomas: a review of morphology and immunophenotypical analysis. Mod Pathol. 2004 Aug 27.

22. Jones TD, Ulbright TM, Eble JN, Baldridge LA, Cheng L. OCT4 staining in testicular tumors: a sensitive and specific marker for seminoma and embryonal carcinoma. Am J Surg Pathol. 2004 Jul; 28(7):935-40.

23. Madjd Z, Spendlove I, Moss R, Bevin S, Pinder SE, Watson NF, et al. Upregulation of MICA on high-grade invasive operable breast carcinoma. Cancer Immun. 2007; 7:17.

24. Madjd Z, Spendlove I, Pinder SE, Ellis IO, Durrant LG. Total loss of MHC class I is an independent indicator of good prognosis in breast cancer. Int J Cancer. 2005 Nov 1; 117(2):248-55.

25. Cheng L, Thomas A, Roth LM, Zheng W, Michael H, Karim FW. OCT4: a novel biomarker for dysgerminoma of the ovary. Am J Surg Pathol. 2004 Oct; 28(10):1341-6.

26. Ezeh UI, Turek PJ, Reijo RA, Clark AT. Human embryonic stem cell genes OCT4, NANOG, STELLAR, and GDF3 are expressed in both seminoma and breast carcinoma. Cancer. 2005 Nov 15; 104(10):2255-65.

27. Sperger JM, Chen X, Draper JS, Antosiewicz JE, Chon CH, Jones SB, et al. Gene expression patterns in human embryonic stem cells and human pluripotent germ cell tumors. Proc Natl Acad Sci U S A. 2003 Nov 11; 100(23):13350-5.

28. Almstrup K, Hoei-Hansen CE, Wirkner U, Blake J, Schwager C, Ansorge W, et al. Embryonic stem cell-like features of testicular carcinoma in situ revealed by genome-wide gene expression profiling. Cancer Res. 2004 Jul 15; 64(14):4736-43.

29. Ruangpratheep C, Lohachittranond C, Poonpracha T, Punyarit P. OCT4 expression on a case of poorly differentiated (insular) carcinoma of the thyroid gland and minireview. J Med Assoc Thai. 2005 Nov; 88 Suppl 3:S281-9.