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The Expression Pattern of Oct4 Stem Cell Marker in Thyroid Cancers among Sudanese Patients

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Abstract:

This study was conducted in Khartoum state to evaluate the expression pattern of Oct4 stem cell marker among Sudanese patients. Many tissues if not all are thought to contain stem cells that are responsible for regeneration and repair of the tissue after injury. Notably, the so-called cancer stem cells or tumor-initiating cells, have been studied in order to understand the mechanisms of carcinogenesis and/or metastasis. However, the nature of cancer stem cells, let alone normal stem/progenitor cells, particularly those of the thyroid remains a gap in knowledge Understanding of the mechanism for thyroid regeneration and mode of participation of normal adult thyroid stem/progenitor cells in this process will hopefully yield a more complete understanding of the nature of thyroid cancer stem cells, and/or help understand the pathogenesis of other thyroid diseases. Oct4 has been consistently associated with pluripotent or stem like cells, and it is hypothesized that Oct4 is necessary for the maintenance of pluripotency. We hypothesize that Oct4-positive cells are present in thyroid cancer cells. To test this hypothesis, 34 cases of thyroid neoplasms were evaluated for Oct4 expression using immunohistochemistry. The results of this study showed that all tumors included in this study contained a subpopulation of Oct4-positive cells, Most of thyroid cancer patients were in the sixth and seventh decants of life. Although the proportion of Oct4-positive cells and the intensity of immunoreactivity varied both within and between tumor types. Subpopulations of Oct4-positive cells identified in these

tumors are likely to represent "cancer stem" cells and therefore might be responsible for the maintenance and propagation of the tumors. If these cells represent cancer stem cells, and are therefore responsible for the maintenance and growth of the neoplastic cellular population, then these cells should serve as relevant therapeutic targets and offer the greatest potential for curative treatment.

Key words: expression pattern Oct4 stem cell marker, thyroid cancers, Sudan

1. INTRODUCTION AND LITERATURE REVIEW

1.1 Thyroid neoplasms:

Thyroid tumors are diseases in which the thyroid cells become abnormal, grow uncontrollably, and form a mass of cells called a tumor. Thyroid cancers are originating from follicular or parafollicular thyroid cells. These cells give rise to both well-differentiated cancers (i.e., papillary and follicular) and anaplastic thyroid cancer. The second cell type, the C or parafollicular cell, produces the hormone calcitonin and is the cell of origin for medullary thyroid carcinoma (MTC)[(1)].

1.2 The epidemiology:

The most common thyroid disease in the community is simple (diffuse) physiological goitre. Ultrasonography has been used in epidemiological studies to assess thyroid size, leading to much higher estimates of goitre prevalence than in studies in which goitre size was assessed by physical examination. The most common thyroid condition is hypothyroidism, or underactive thyroid[(2)]. In the United States, hypothyroidism usually is caused by an autoimmune response known as Hashimoto's disease or autoimmune thyroiditis. As with all autoimmune diseases, the body mistakenly identifies its own tissues as an invader and attacks them until the organ is destroyed. This chronic attack eventually prevents the thyroid from releasing

adequate levels of the hormones T3 and T4, which are necessary to keep the body functioning properly. The lack of these hormones can slow down metabolism and cause weight gain, fatigue, dry skin and hair, and difficulty concentrating. annually incidence rates vary by geographical locations age and sex the age adjusted annual incidence (from 1996 to2000) in the United States 68 new cases per million (2.3) with a higher incidence in women (99/million) than men (36/million)(2.4) approximately 25.690 new cases of thyroid cancer are now diagnosed annually in the United States with the females male ratio close to 3:1[3]. Based on recent data, thyroid cancer is the fifth most common cancer in women, and in Italy, it is the second most frequent cancer in women below 45 years of age [4]. Only in few countries (Norvay, Sweden) thyroid cancer incidence is decreased according to the National Cancer Institute, there are about 56,000 new cases of thyroid cancer in the US each year, and the majority of those diagnoses are papillary thyroid cancer the most common type of thyroid cancer. Females are more likely to have thyroid cancer at a ratio of 3:1. Thyroid cancer can occur in any age group, although it is most common after age 30, and its aggressiveness increases significantly in older patients. Thyroid cancer does not always cause symptoms; often, the first sign of thyroid cancer is a thyroid nodule. Some thyroid cancer signs and symptoms include a hoarse voice, neck pain, and enlarged lymph nodes. Although as much as 75% of the population will have thyroid nodules, the vast majority are benign. Thyroid cancer, in 2010, resulted in 36,000 deaths globally up from 24,000 in 1990[5]. Obesity may be associated with a higher incidence of thyroid cancer, but this relationship remains the subject of much debate.[6] In Western Sudan over 8 years One hundred and twelve patients with thyroid malignancy seen at The Radio-isotope Centre, Khartoum (RICK) during the period 1982-1989 were studied. The female to male ratio was 2.5:1.0 with a high incidence of the disease between the ages of 40 and

70 years. Follicular carcinoma was the commonest (42%) followed by papillary (22.3%) and anaplastic (21.4%). Goitre was the main presenting symptom (92.9%)Thyroid disorders are common worldwide.[7].In Africa, dietary iodine deficiency is the major determinant of thyroid pathology, resulting in a spectrum of iodine deficiency disorders, including goitres, hypothyroidism and mental retardation.[8] Of these, mental retardation poses the most severe threat to socioeconomic wellbeing; thus, its prevention has been the focus of current global efforts towards sustainable iodine sufficiency. [9] At least 350 million Africans are at risk of iodine deficiency.[10] According to World Health Organization (WHO) estimates, goitres are present in 28.3% of the African population, [10] and approximately 25% of the global burden of iodine deficiency as measured by disability-adjusted life years (DALYs) occurs in Africa. [11] However, recent decades have seen remarkable improvements in iodine nutrition through salt iodination in the continent.[12] The effect of these developments on the pattern of thyroid gland disease are beginning to unravel and will be relevant to the strategies for extending the present gains. At El Obeid Hospital, Western Sudan; thyroidectomy was commonly performed in female patients (87.8%) and the majority of patients (74.8%) were between 20 and 50 years old. [13]

1.3 Risk factors of thyroid tumors:

A risk factor is anything that increase your chance of getting a disease like cancer includes:-

1.3.1 Exposure to radiation:

Ionizing radiation is considered the predominant risk factor for inducing thyroid cancer. The thyroid gland is sensitive to external and internal radiation and strong dose-response relationship between the incidence of thyroid cancer and radiation absorbed dose has been reported .The most common thyroid manifestation of radiation is hypofunction, as well as

thyroid nodules and thyroid cancer. Autoimmune thyroid disease has been linked to therapeutic medical radiation [14] [15]..

1.3.2 Environmental Toxicants:

A wide range of environmental toxicants have been identified that interfere with thyroid hormone production, metabolism, and action. Most of these agents, at sufficient doses, interfere with thyroid function and their effect can be detected by an elevation in serum TSH or a reduction in serum thyroxine (T4) or T3. It is now recognized, however, that a number of these agents may also interfere with the hypothalamic–pituitary–thyroid regulatory axis and be associated with a reduced serum T4 or T3 concentration, but a normal range TSH [16] [17].

1.3.3 Genetic background:

Certain inherited genetic abnormalities have been associated with the development of different types of thyroid cancer. Given that 70%–80% of susceptibility to autoimmune thyroid disease is based on genetics, individuals with a personal history of autoimmune disease or family history of autoimmune thyroid disease are the most susceptible. Those with a sibling that has autoimmune thyroid disease are at increased risk, especially strong for Hashimoto's thyroiditis [18].

1.3.4 Cigarette smoking:

Cigarette smoking, as well as cessation of smoking, have been linked to the onset of autoimmune thyroid disease. The increase in risk of the onset of autoimmune thyroid disease with cessation of smoking may be useful in monitoring susceptible patients who stop smoking for the myriad health benefits. For example, cessation of smoking may be associated with weight gain, and hypothyroidism should be considered as a cause. Cigarette smoke contains cyanide, which is metabolized to thiocyante, and can interfere with iodine concentration in the thyroid and in the lactating breast [19].

1.3.5 Weight:

It's imperative dietitians have a good understanding of the metabolic changes associated with thyroid disease so they can set realistic goals and expectations for clients. Most people with hypothyroidism tend to experience abnormal weight gain and difficulty losing weight until hormone levels stabilize. Moreover, it's common for patients with Graves' disease to experience periods of high and low thyroid hormone levels, so it may take several months to achieve a balance. During this time, it's essential clients focus on healthful behaviors such as eating nutritious foods, exercising regularly, managing stress, and sleeping adequately rather than focus on the numbers on the scale. Clara Schneider, MS, RD, RN, CDE, LDN, of Outer Banks Nutrition and author of numerous books, including *The Everything Thyroid Diet Book*, says, "The No. 1 priority is to get the thyroid disease under control. Clients need to have labs and medications addressed first. Weight changes are just not going to happen before all of that is under control." She notes that Hashimoto's typically occurs around menopause, which compounds the weight gain issue that many women experience during that time.

1.3.6 Iodine intake:

Iodine is a vital nutrient in the body and essential to thyroid function. Thyroid hormones are comprised of iodine. While autoimmune disease is the primary cause of thyroid dysfunction in the United States, iodine deficiency is the main cause of the reduction of thyroid hormone production, regular and adequate iodine intake is optimal for thyroid and reduces susceptibility to agents that influences the thyroid by interfering with iodine uptake ,such as perchlorate. (20)

1.3.7 Gender and age:

For unclear reasons thyroid cancers (like almost all diseases of the thyroid) occur about 3 times more often in women than in

men. Thyroid cancer can occur at any age, but the risk peaks earlier for women (who are most often in their 40s or 50s when diagnosed) than for men (who are usually in their 60s or 70s). Although thyroid cancer can occur in people of all ages, most cases diagnosed with thyroid cancer are between the ages of 20 and 60[21].

1.3.8 Dietary intake:

The evidences of a possible effect of nutrient/food or environmental pollutants on thyroid cancer are weak and not confirmed. Studies aimed at identifying cancer risk factors belonging to diet and lifestyle have provided controversial results because food and drinks have a great number of different constituents (many unmeasured or highly variable) and also because dietary intake and lifestyle may significantly change in the same individual over time[22].

1.4 Types and classification of thyroid cancer:

1.4.1 Papillary thyroid cancer:

Papillary carcinoma (PTC) is the most common form of well-differentiated thyroid cancer, and the most common form of thyroid cancer to result from exposure to radiation, accounting for about 80% of thyroid cancers. While papillary thyroid cancer typically occurs in only one lobe of the thyroid gland, it may arise in both lobes in up to 10% to 20% of cases. Papillary carcinoma appears as an irregular solid or cystic mass or nodule in a normal thyroid parenchyma .Papillary thyroid cancer is most common in women of childbearing age. [23]. Even though papillary thyroid cancer is usually not an aggressive type of cancer, it often metastasizes (spreads) to the lymph nodes in the neck. Papillary thyroid cancer treatment usually is successful.

1.4.2 Follicular thyroid cancer:

It accounts for about 10% of thyroid cancers. Like papillary thyroid cancer, follicular thyroid cancer usually grows slowly. Its outlook is similar to papillary cancer, and its treatment is the same. Follicular thyroid carcinoma (FTC) is a well-differentiated tumor. In fact, FTC resembles the normal microscopic pattern of the thyroid. FTC originates in follicular cells and is the second most common cancer of the thyroid, after papillary carcinoma. Follicular and papillary thyroid cancers are considered to be differentiated thyroid cancers; together they make up 95% of thyroid cancer cases. [24]. Follicular thyroid cancer usually stays in the thyroid gland but sometimes spreads to other parts of the body, such as the lungs or bone. However, it usually does not spread to lymph nodes. It is more common in countries where diets do not contain enough iodine.

1.4.3 Medullary thyroid cancer (MTC) :

This is the only type of thyroid cancer that develops in the parafollicular cells of the thyroid gland. It accounts for 3% to 10% of thyroid cancers. Medullary cancer cells usually make and release into the blood proteins called calcitonin and/or carcinoembryonic antigen, which can be measured and used to follow the response to treatment for the disease.[25]

1.4.4 Anaplastic thyroid cancer:

This is the most dangerous form of thyroid cancer. It is makes up only 1% of thyroid cancers. It is believed that anaplastic thyroid cancer grows from a papillary or follicular tumor that mutates further to this aggressive form. Anaplastic thyroid cancer spreads rapidly into areas such as the trachea, often causing breathing difficulties. Anaplastic carcinoma (ATC) is a very rare form of thyroid cancer. Although sharing some characteristics with papillary disease, it is thought to develop from an existing follicular cancer that further mutated, that is, became undifferentiated over time. It is believed that

anaplastic cancers are likely long existing tumours that were left untreated and suddenly became aggressive. This form of cancer spreads rapidly and is much harder to treat. About 1.5% of cases are anaplastic. [26].

1.5 Staging of thyroid cancer:

Staging refers to the process of determining how severe the cancer and the stage helps to determine the prognosis (i.e. the chance that a patient will recover or die of a disease). There are many staging systems for predicting the outcome of thyroid cancer. These staging systems look at various characteristics of the cancer as well as the patient.

The TNM method is the most universally used staging method and applies to both papillary and follicular thyroid cancers.

It was introduced in 1987 by the International Union against Cancer and adopted by the American Joint Commission on Cancer.

TNM stands for Tumor/Node Metastasis/Distant Metastasis:

TNM Classification for Thyroid Cancer

The TNM classification for thyroid cancer is provided below

Table. 1.1

Primary tumor (T)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor is found
T1	Tumor size ≤ 2 cm in greatest dimension and is limited to the thyroid
T1a	Tumor ≤ 1 cm, limited to the thyroid
T1b	Tumor > 1 cm but ≤ 2 cm in greatest dimension, limited to the thyroid
T2	Tumor size > 2 cm but ≤ 4 cm, limited to the thyroid.
T3	Tumor size >4 cm, limited to the thyroid or any tumor with minimal extrathyroidal extension (eg, extension to sternothyroid muscle or perithyroid soft tissues)
T4a	Moderately advanced disease; tumor of any size extending beyond the thyroid capsule to invade subcutaneous soft tissues, larynx, trachea, esophagus, or recurrent laryngeal nerve
T4b	Very advanced disease; tumor invades prevertebral fascia or encases carotid artery or mediastinal vessel

All anaplastic carcinomas are considered stage IV:

T4a Intrathyroidal anaplastic carcinoma

T4b Anaplastic carcinoma with gross extrathyroid extension

Regional lymph nodes (N) N: Lymph Nodes

- NX: regional lymph nodes can't be assessed
- N0: no involved regional lymph nodes
- N1: involved regional lymph nodes
    - N1a: involved central neck lymph nodes
    - N1b: involved lateral neck or mediastinal (chest) lymph nodes

Distant metastasis (M)

M0 No distant metastasis is found

M1 Distant metastasis is present

Stage 1 is the least advanced form of cancer with the best prognosis, and Stage 4 is the most advanced category. The table below shows the likelihood of a local recurrence (i.e. recurrence of thyroid cancer in the neck region), distant recurrence (i.e. recurrence of cancer in other areas of the body), and mortality (i.e. death) based on the stage of a given tumor for well-differentiated thyroid cancers in general.(26) for patients with well-differentiated thyroid cancer (i.e. papillary, follicular, and Hurthle cell cancer), age is the most important prognostic factor. If a patient is younger than 45, even if there are distant metastases, they are considered a Stage II and have an excellent prognosis. For patients with medullary thyroid cancer, age is not an important prognostic factor .(27)

Stage of thyroid cancer(table 1.1)

Separate stage groupings are recommended for papillary or follicular (differentiated), medullary, and anaplastic (undifferentiated) carcinoma

Papillary and follicular thyroid cancer (age < 45y):

Stage	**T**	**N**	**M**
I	Any T	Any N	M0
II	Any T	Any N	M1

Papillary and follicular; differentiated (age ≥ 45y):

Stage	**T**	**N**	**M**
I	T1	N0	M0

II	T2	N0	M0
III	T3	N0	M0
IVA	T1-3	N1a	M0
	T4a	N1b	M0
IVB	T4b	Any N	M0
IVC	Any T	Any N	M1
Anaplastic carcinoma (all anaplastic carcinomas are considered stage IV):			
Stage	**T**	**N**	**M**
IVA	T4a	Any N	M0
IVB	T4b	Any N	M0
IVC	Any T	Any N	M1
Medullary carcinoma (all age groups):			
Stage	**T**	**N**	**M**
I	T1	N0	M0
II	T2, T3	N0	M0
III	T1-T3	N1a	M0
IVA	T4a	N0	M0
	T4a	N1a	M0
	T1	N1b	M0
	T2	N1b	M0
	T3	N1b	M0
	T4a	N1b	M0
	T4a	N0, N1b	M0
	T1-T4a	N1b	M0
IVB	T4b	Any N	M0
IVC	Any T	Any N	M1

1.6 Diagnosis of thyroid cancer:

Robust diagnostic facilities for thyroid disorders are lacking in most countries in Africa and the commonly employed diagnostic techniques include immunoassays, serology, ultrasonography cytology, and histopathological techniques for the evaluation of thyroid nodules.

Computed tomographic scans and magnetic resonance imaging facilities are also not widely available but when available are often inaccessible for most patients because of the

system of health care provision which is often that of "out of pocket" payment.

Fine needle aspiration cytology (FNAC) is commonly employed in the evaluation of thyroid nodules in the African continent and in Sudan, usually patients presenting with nontoxic goiters are made to undergo FNAC. A Tunisian report[28].noted that the interpretability rate of FNAC in the evaluation of thyroid nodules was 7.52%, sensitivity as compared with that of histopathology was 70% and a specificity of 97.43%. In a Nigerian series, the diagnostic accuracy of FNAC for malignancy was reported to be 80.6% with a sensitivity and specificity 83% and 80%, respectively. [29].The actual diagnosis of thyroid cancer is made from the results of a biopsy, in which cells from the suspicious area are removed and looked at under a microscope.

1.7 Cancer stem cells:

These refer to a subset of tumor cells that has the ability to self-renew and generate tumor heterogeneity. For the past several years, a number of studies have characterized adult normal thyroid stem/progenitor cells and thyroid cancer stem cells, the latter using various human thyroid tumors and tumor cell lines to determine the mechanisms of thyroid carcinogenesis and/or metastasis [30] The nature of thyroid cancer stem cells is poorly understood. For instance, it is not known whether cancer stem cells are the result of thyroid stem cells acquiring mutations or through epithelial–mesenchymal transition, or a small portion of cancer cells acquiring properties of stem cells following dedifferentiation or through other mechanisms[31]. Alternatively, fetal thyroid cell carcinogenesis theory suggests that cancer cells are directly generated from fetal cells [32]. In order to address these unresolved questions and to understand the nature and/or role of cancer stem cells in thyroid carcinogenesis and/or metastasis, characterization of adult normal thyroid stem/progenitor cells, if present, is of great

importance.Many tissues if not all are thought to contain stem cells that are responsible for regeneration and repair of tissue after injury.

1.7.1 Theory of cancer stem cells:

Cancer is characterized by mutations that cause uncontrolled cell proliferation and the formation of tumors. Although the vast majority of these mutations activate cell cycle checkpoints that curtail hyperproliferation, there are instances in which cells escape these checkpoints and develop into cancer. Some evidence suggests that a small population of tumor cells have stem cell like properties. This has led to the evolution of the cancer stem cell hypothesis. This theory states that tumors both initiate and are maintained by this small population of cancer stem cells. It is uncertain whether these cells are actually stem cells, or if they are formerly normal cells that have obtained stem cell like properties. If these cells do originate from stem cells, it will be important to determine whether they are stem cells or progenitor cells.

The cancer stem cell hypothesis arises in part from the observation that cancer cell populations are not homogenous. In 1971, Park et al. [33]was able to show that although tumors arise from a single cell, the cells that constitute the tumor are not identical to one another. A side population was identified among thyroid cancer cell lines for the first time in 2007; 0.25% of cells in the anaplastic thyroid carcinoma cell line were determined to be side-population cells. The cancer stem cell theory couples the idea that stem cells are responsible for cancer with the hypothesis that distinct mutations in signaling pathways are involved in tumorgenicity. Signaling pathways with ES cell proliferation and differentiation are particularly important to the cancer stem cell theory. [34]

1.7.2 Cellular origin of thyroid cancer stem cells:

The thyroid cancer stem cell hypothesis holds that thyroid cancer stem cells originate either from normal stem cells, progenitor cells, or more mature cells that have dedifferentiated. Although any of these origins is possible, most researchers believe that stem cells or progenitor cells are the most likely culprits. Cancer progression requires that cells overcome the barrier that somatic cells have in regard to proliferation, and the lifespan of differentiated cells is too short to obtain all the mutations associated with cancer. The primary evidence for this theory is that cancer populations are not homogenous. Numerous studies have shown that only a subset of cancer cells – those with properties of stem cells are tumorigenic. Although the discovery of stem cell markers in the thyroid gland indicates the potential for the existence of thyroid cancer stem cells, the identification of these cells could prove to be quite difficult due to the extremely low lifetime turnover in the thyroid gland.The cellular origin of anaplastic carcinoma is of special interest because no successful treatment for it exists. The classical view for its origin is that it results from additional mutations to papillary carcinomas [(35)]. However, while papillary carcinomas are marked with rearrangement of the RET gene, these mutations are not generally found in anaplastic carcinomas. To date, there has been no confirmation that anaplasticcarcinoma does result from additional mutations to papillary carcinomas.Takano& Amino (2005) use this evidence to support their hypothesis for the origin of anaplastic carcinoma. Their point of view is that anaplastic carcinoma forms from the remnants of fetal thyroid cells, instead of normal thyroid follicular cells, before adolescence, and already have cancer properties prior to the onset of their division. Furthermore, the fetal cell carcinogenesis hypothesis suggests a similar gene expression profile between fetal thyroid cells and thyroid cancer cells [(36)].

1.7.3 Oct4 stem cell marker:

Oct-4 (octamer-binding transcription factor4) also known as POU5F1 (POU domain, class 5, transcription factor 1) is a protein that in humans is encoded by the *POU5F1* gene[37].

This protein is critically involved in the self-renewal of undifferentiated embryonic semcells [38].Oct4 is expressed in embryonic stem (ES) cells, and their over-expression can induce pluripotency in both mouse and human somatic cells, indicating that these factors regulate the developmental signaling network necessary for ES cell pluripotency[39].Oct4 is a major transcription factor that is mandatory for the self-renewal and pluripotency characteristics of ES cells and germ cells. Rare cells that express Oct4 were identified in several somatic cancers [39].

Oct-4 transcription factor is initially active as a maternal factor in the oocyte but remains active in embryos throughout the preimplantation period. Oct-4 expression is associated with an undifferentiated phenotype and tumors. Oct4 is a major transcription factor that is mandatory for the self-renewal and pluripotency characteristics of ES cells and germ cells. Rare cells that express Oct4 were identified in several somatic cancers [40]. Oct4A expressing cells are present in human benign and malignant prostate glands and the frequency of Oct4A expressing cells increases in prostate cancers [40]. A subpopulation of the Oct4A expressing cells co-expressed Sox2, an ES cell marker. In the intestine, Oct4 expression causes dysplasia by inhibiting cellular differentiation in a manner similar to that in the ES cells[41].

1.8 Treatment options thyroid cancer:

1.8.1 Chemotherapy: uses anti cancer (cytotoxic) drugs to destroy cancer cell and it depends on stage of cancer.

1.8.2 Radiotherapy: its shrink tumors and reduce symptom and the doctor use radiotherapy to treat ovarian cancer that has spread to another organ in the body.

1.8.3 Surgery: Thyroidectomy and dissection of central neck compartment is initial step in treatment of thyroid cancer in majority of cases[42].

1.9 Thyroid Cancer Prognosis:

Most thyroid cancers are very curable. In fact, the most common types of thyroid cancer (papillary and follicular thyroid cancer) are the most curable. In younger patients, both papillary and follicular cancers have a more than 97% cure rate if treated appropriately. Both papillary and follicular thyroid cancers are typically treated with complete removal of the lobe of the thyroid that harbors the cancer.

The argument against early diagnosis and treatment is based on the logic that many small thyroid cancers (mostly papillary) will not grow or metastasize. This viewpoint holds the overwhelming majority of thyroid cancers are overdiagnosed (that is, will never cause any symptoms, illness, or death for the patient, even if nothing is ever done about the cancer). Including these overdiagnosed cases skews the statistics by lumping clinically significant cases in with apparently harmless cancers[43]. Thyroid cancer is incredibly common, with autopsy studies of people dying from other causes showing that more than one third of older adults technically has thyroid cancer, which is causing them no harm. [44]. Medullary thyroid cancer is significantly less common but has a worse prognosis. Medullary cancers tend to spread to large numbers of lymph nodes very early on, and therefore require a much more aggressive operation than the more localized thyroid cancers, such as papillary and follicular thyroid cancer. Thyroid cancer is three times more common in women than in men, but according to European statistics,

(45).the overall relative 5-year survival rate for thyroid cancer is 85% for females and 74% for males. (46).Prognosis is better in younger people than older ones.(44 Prognosis depends mainly on the type of cancer and cancer stage.

2. RATIONALE

Thyroid cancer is the most frequently diagnosed endocrine cancer and causes more deaths than all other endocrine cancers combined .the role played by Oct4 protein in human embryonic stem cell self-renewal remains unclear. There is no doubt that the thyroid gland retains a significant number of resident stem cells as shown in mice and human thyroid tissue but the role of thyroid cancer stem cells (CSCs) in tumor formation and progression is still a gap in knowledge, this work may help in better diagnosis and management of the patients.

3. OBJECTIVE:

3.1. General objective:

To observe the expression of OCT4 stem cell marker in thyroid neoplasms.

3.2 Specific objectives:

- To detect OCT4 marker expression in different types thyroid cancers.
- To detect the correlation of the age and OCT4 expression.
- To detect the relation between the gender and the expression of OCT4 marker.

4. MATERIALS AND METHODS

4.1 Study design

This is non-interventional case study.

4.2 Study area
This study was conducted at Al Zaeim Alazhari University, Khartoum, Sudan

4.3 Study population
Previously diagnosed ovarian cancer patients

4.4 Ethical considerations
An ethical permission was obtained from relevant authorities.

4.5 Sampling

4.5.1 Data collections
The data have being collected from different Labs records by retrieving both request forms and microscopically examination results.

4.5.2 Sample size
34 samples were collected in this study.

4.5.3 Sample type
Previously diagnosed archival formalin fixed- paraffin embedded blocks.

4.6 Study period
The studies were carried out during period from May to December 2015.

4.7 Sampling technique

4.7.1 Block preparation for tissue microarray
Target area from origin was identified on the H& E ready stained sections using permanent marker so that the corresponding area on the tissue block can be sampled. Origin block was then subjected to 3mm skin punch (Miltex biopsy

punch, Germany) and tissue was carefully punched. The selected core was then brought in to recipient paraffin block. The surface of TMA blocks were then pressed by preheated clean glass slide until the surface became smooth, then blocks were placed in refrigerator until cooling. Glass slide was then detached and the block was ready for cutting.

4.7.2 Sectioning:

Tissue microarray block was sectioned by using Rotary microtome (Leica RM 2125) and low profile disposable Knives by using 4 micron as thickness of choice.

Sections were then floated on a floating water bath adjusted to 45℃. Finally clean coated glass slides in addition to ordinary slides were used to pick up the floated section and slides were left in a 60℃ for 2 hours.

4.8 Staining protocols:

4.8.1 Haematoxylin and eosin

Sections were dewaxed in xylene and rehydrated through graded alcohol to distilled Water. The slides were then placed in Mayer Haematoxylin solution for 10Minutes, washed in tap water for 5 minutes and counterstained with Eosin solution for 3 minutes. The slides were dehydrated through alcohols, cleared in xylene and Mounted in DPX mounting media.

4.8.2 Immunohistochemistry protocol

4.8.2.1 Antigen retrieval

The slides and positive controls were dewaxed in Xylene, dehydrated through graded alcohols to distilled water. Slides were then placed in preheated buffer (ProTaqs® Suprema-Germany, antigen enhancer pH9.0 prepared 1 in 50 v/v) at 96^{o} C in a water bath for 40 minutes. After completion of the

retrieval the coplin jar that contained the slides were removed from water bath and allowed to cool to room temperature.

4.8.2.2 Staining technique:

The Staining procedures were carried out by using ProTaqs® Suprema Polycolor3 Anti-mouse/ Anti-Rabbit IgG/ HRP DAB liquid kits as followings:

After slides reached the room temperature, slides were washed in TBS buffer with Tween 20, pH 7.6 for 2 minutes. After that a circle made around the sections by using Dako pen (Dako Denmark A/S). The sections were then covered with primary antibodies for E-cadherin (clone 156-3C11, Thermo scientific) for 50 minutes. Section washed in TBS buffer with Tween 20, pH7.6 for 2 min, endogenous peroxidase enzymes was then blocked by 3% hydrogen peroxide for 10 minutes, washed in TBS buffer for 5min, and the primary antibody enhancer applied on the sections for 10 min. Slides then were washed in TBS buffer for 5min, covered by anti-rabbit/anti-mouse IgG- polymer HRP for 10 min and washed in TBS for 10 min. The DAB chromogen was then applied on to the slides (1ml substrate buffer+ 1drop of DAB chromogen). After that sections were washed in distilled water and counterstained with Mayer's Haematoxylin for 1 min, washed in distilled water and left to air dry for 5min. Finally slides were cleared in xylene and mounted with a cover glass using DPX mounting media.

4.9 Microscopic examination

Using Light microscopy, the section were examined under different magnification powers, low (4X), medium (10X) and high (40X), for tissue and cellular changes and Octamer4 expression.

5. RESULTS:

5.1 Samples recruitment and histological types

Thirty four cases of thyroid tumor were included in this study. These were contained in 2 tissue microarray blocks.

The frequency of histological types was as follow:13 cases of papillary carcinoma,11 cases of follicular carcinoma,34 cases of anaplastic carcinoma, 2 cases of undifferentiated and poorly differentiated tumor, 2 cases of hyper plastic goitrous nodule, 1 case of follicular adenoma.

5.2 Patient age:

The patients' age ranged between 14 and 78 years with mean age of about 50 years.

5.3 Oct4 expression pattern

The oct4 showed positive staining reaction in 27 cases and negative in 7 cases which distributed as following: 3 cases of follicular carcinoma,2 cases of anaplastic carcinoma, 1 case of undifferentiated tumor. (fig5.2)

5.4 Relation between tumor types and patient age:

The P value was (0.890) which is statistically insignificant that means there is no clear relation between the expression of Oct4 and the age of patient.

5.5 Relation of sex to the expression of Oct4:

The P value was (0.068) which is statistically insignificant that means there is no obvious relation between the expression of Oct4 and the gender of patient. (Table 5.2)

Relation between types of thyroid cancer and Oct4 expression (table 5.1)

		octc4		Total
		Positive	Negative	
Cancer	Papillary	13	0	13
	Follicular	8	2	10
	Ananplastic	3	1	4
	un+poor	1	2	3
	Benign	1	2	3
Total		27	7	34

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	11.471[a]	4	.022
Likelihood Ratio	12.430	4	.014
Linear-by-Linear Association	10.489	1	.001
N of Valid Cases	34		

P value = 0.022 which statically significant.

Gender relation to octc4 relation
(table 5.2)

		octc4		Total
		Positive	Negative	
Sex	Male	9	5	14
	Female	18	2	20
Total		27	7	34

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.331[a]	1	.068
Continuity Correction[b]	1.943	1	.163
Likelihood Ratio	3.322	1	.068
Fisher's Exact Test			
Linear-by-Linear Association	3.233	1	.072
N of Valid Cases	34		

P value = 0.068 which statically insignificant

Age relation to oct 4(table 5.3)

		octc4		Total
		Positive	Negative	
Age	10-20	1	0	1
	21-30	1	1	2
	31-40	4	1	5
	41-50	4	0	4
	51-60	6	2	8
	61-70	6	2	8
	71-80	2	0	2
	44.00	2	1	3
	55.00	1	0	1
Total		27	7	34

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.622[a]	8	.890
Likelihood Ratio	4.984	8	.759
Linear-by-Linear Association	.015	1	.901
N of Valid Cases	34		

P value = 0.890 which statically insignifacant

Fig 5.1

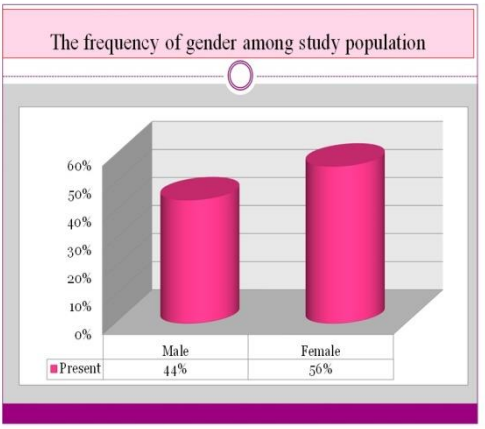
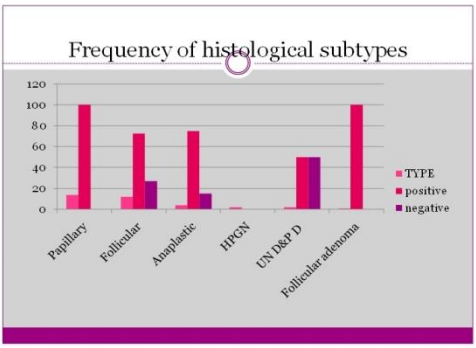


Fig5.2:



5.6 Samples and histological types:

Thirty four cases of thyroid tumor were included in this study. These were contained in 2 tissue microarray blocks.

The frequency of histological types was as follow: 13 cases of papillary carcinoma,11 cases of follicular carcinoma,34 cases of anaplastic carcinoma,2 cases of undifferentiated and poorly differentiated tumor,2 cases of hyper plastic goitrous nodule, 1 case of follicular adenoma.

6. DISCUSSION:

This study was carried out in Khartoum State to detect the pattern of Oct 4 expression in thyroid cancers among Sudanese patients. Oct4-expressing subpopulations of neoplastic cells were identified in every tumor included in this study.

Thirty four cases of thyroid tumors were included: in Papillary thyroid cancer 13 cases were all stained positive for Oct4 stem cell marker; in Follicular thyroid carcinoma 12 cases were included only 3 were negatively stained; in anaplastic thyroid cancer 4 cases were included only 1 case was negatively stained, in Hyper plastic goitrous nodule 2 cases were included and all stained positive, in Undifferentiated and poorly differentiated 2 cases were stained one showed negative, only one case of Follicular adenoma was included and was positive.

These findings agreed with study done by Carina V et al 2013 [47]. furthermore in a study conducted by J. D. Webster el al in which all of the 83 tumors included in his study expressed Oct4 in a subpopulation of neoplastic cells. And the pattern of Oct4 expression varied both in the intensity of nuclear staining and in the proportion of Oct4-positive cells within a tumor. [48].

Concerning the relation between gender and Oct4 expression, thyroid cancer was common in female patients than in men and the majority of patients were between 60 And 70 years old the expression of Oct 4 had no obvious relationship with the patients age and gender .their results was statistically insignificant (P value 0.068).

These results were agree to Wang H[1], Wang J., et.al (2010) who reported that Oct-4 expression was observed in all the thyroid-related diseases . In thyroid papillary carcinomas, the expression of Oct-4 were higher than that in thyroid adenoma, and had no clear relationship with the patients age, sex, the size and location of tumor and tumor metastasis.

And there are more stem cells in medullary thyroid carcinomas and follicular carcinomas. [49]. The proportion and distribution of Oct4-positive cells varied both within and between tumor types; however, these cells were seen consistently within all of the tumors. Based on the association of Oct4 with stem cells and the maintenance of an undifferentiated state and pluripotency, we hypothesize that these subpopulations of Oct4-positive cells might represent subpopulations of “cancer stem” cells or progenitor cells that are responsible for the maintenance and endless proliferative capacity of the respective neoplasm. [50] [51].

The variation in Oct4 expression within and between tumor types most likely represents variation in the cellular differentiation of neoplastic subpopulations. In cancer, tumors are made up of clonal subpopulations of neoplastic cells that are originally derived from a single cell. Within a given tumor, each of these clonal subpopulations might be blocked at unique

stages of cellular differentiation, leading to phenotypic variations[52) (53)].

Therefore, depending on the degree of differentiation and the point at which differentiation is blocked, some cells that are blocked early in cellular differentiation might maintain high levels of Oct4, whereas other cells that are able to partially differentiate might do so to a degree that Oct4 is down regulated. In some tumors, most daughter cells might undergo partial differentiation, which results in Oct4 down regulation in the majority of cells, whereas only the true cancer stem cell population, which is responsible for the long-term maintenance of the cancer, continues to express Oct4. The latter might account for the rare randomly scattered Oct4-positive cells seen in many of the tumors examined.

Previous studies have reported variable Oct4 expression in human tumors, often finding little to no Oct4 expression in the majority of tumors[54) (55)]

Therefore, depending on the methods used for immunohistochemical evaluation and the criteria used to consider a tumor positive for Oct4, several tumors might have been considered Oct4 negative when, in fact, Oct4-positive cells were present within the neoplastic tissue. Additionally, variation in the antibodies and immunolabeling conditions might have further resulted in discrepancies between studies. However, further studies are needed to better characterize these cells and to define their role in tumorigenesis. At present, little is known as to which cells are the true progenitor cells for cancer and how these progenitors might be targeted in order to improve the therapeutic response to a given tumor. If these Oct4-positive cells truly represent cancer stem cells, they might serve as the best target for eliminating many forms of cancer.

7. CONCLUSIONS AND RECOMMENDATIONS:

7.1 Conclusions:

- The expression of Oct4 has been associated with grade of thyroid cancer may be used as an indicator for tumor progression and prognosis
- Most of thyroid cancer patients were in the sixth and seventh decants. Papillary carcinoma is predominant, followed by follicular carcinoma.
- Oct-4 stem cell marker expression was detected in all the thyroid cancers mentioned. In thyroid papillary carcinomas, the expression of Oct-4 were higher than that in thyroid follicular carcinoma, and had no obvious relationship with the patients age and gender.

7.2 Recommendations:

- Much larger population studies and researches on a variety of tumor markers in order to obtain results with high statistical significance to pave the way for inhibitors of these tumors pathways.
- Current drugs on the market have been used in treatment of papillary and anaplastic cancer with promising results. Continuing researches will elucidate the role molecular and histochemical not only to the diagnosis of thyroid cancer ,but also aid in predicting it is course in the individual patient and thus allowing for more directed and targeted treatment.

REFERENCES:

1.Carling, T.; Udelsman,R. (2014). "Thyroid Cancer". Annual Review of Medicine 65: 125–137. doi:10.1146/annurev-med-061512-105739.

2.Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. (Dec 15, 2012). "Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010". Lancet 380 (9859): 2095–128. doi:10.1016/S0140-6736(12)61728-0. PMID 23245604.

3. Santini F, Marzullo P, Rotondi M, Ceccarini G, Pagano L, Ippolito S, Chiovato L, Biondi B (October 2014). "Mechanisms in Endocrinology: The crosstalk between thyroid gland and adipose tissue: signal integration in health and disease". Eur J Endocrinol 171 (4): R137–52. doi:10.1530/eje-14-0067. PMID 25214234

4. Bakheit MA, Mahadi SI, Ahmed ME.Indications and outcome of thyroid gland surgery in Khartoum Teaching Hospital.Khartoum Medical Journal. 2008; 1: 34-37

5. Eltom MA.Endemic goitre in the Sudan. Doctoral Thesis.Uppsala, Sweden:UppsalaUniversity,1984.

6.Takeda J, Seino S, Bell GI (Sep 1992). "Human Oct3 gene family: cDNA sequences, alternative splicing, gene organization, chromosomal location, and expression at low levels in adult tissues". Nucleic Acids Research20 (17): 4613–20. doi:10.1093/nar/20.17.4613. PMC 334192. PMID 1408763

7.Young Lab- Core Transcriptional Regulatory Circuitry in Human Embryonic Stem Cells at MIT

8. Hetzel BS. Iodine deficiency disorders (IDD) and their eradication. Lancet 1983;2: 1126-9

9. P. Sotomayor, A. Godoy, G.J. Smith, and W.J. Huss, Oct4A is expressed by a subpopulation of prostate neuroendocrine cells. Prostate (2008).

10. K. Hochedlinger, Y. Yamada, C. Beard, and R. Jaenisch, Ectopic expression of Oct-4.

11. Mitsutake N, Iwao A, Nagai K, Namba H, Ohtsuru A, Saenko V, et al. Characterization of side population in thyroid cancer cell lines: cancer stem-like cells are enriched partly but

not exclusively. Endocrinology (2007) 148(4):1797–80310.1210/en.2006-1553
12. Lan L, Luo Y, Cui D, Shi BY, Deng W, Huo LL, et al. Epithelial-mesenchymal transition triggers cancer stem cell generation in human thyroid cancer cells. Int J Oncol (2013) 43(1):113–2010.3892/ijo.2013.1913
13. Takano T. Fetal cell carcinogenesis of the thyroid: a modified theory based on recent evidence [My Opinion]. Endocr J (2014).10.1507/endocrj.EJ13-0517 .
14. Hypothyroidism. Bethesda, MD: National Endocrine and Metabolic Diseases Information Service, US Dept of Health and Human Services; 2012. NIH Publication No. 12–6180
15. Jemal, R. Siegel, J. Xu, and E. Ward, "Cancer statistics, 2010," CA: A Cancer Journal for Clinicians, vol. 60, no. 5, pp. 277–300, 2010. View at Publisher · View at Google Scholar · View at Scopus
16.Miller MD. Crofton KM. Rice DC. Zoeller RT. Thyroid-disrupting chemicals: interpreting upstream biomarkers of adverse outcome. Environ Health Perspect. 2009;117:1033–1041. [PMC free article][PubMed]
17. Pearce EN.Braverman LE. Environmental pollutants and the thyroid.Best Pract Res ClinEndocrinolMetab. 2009;23:801–8139.
18. Villanueva R. Greenberg DA. Davies TF. Tomer Y. Sibling recurrence risk in autoimmune thyroid disease. Thyroid.2003;13:761–764. [PubMed]
19.W. J. Mack, S. Preston-Martin, L. Dal Maso et al., "A pooled analysis of case-control studies of thyroid cancer: cigarette smoking and consumption of alcohol, coffee, and tea," Cancer Causes and Control, vol. 14, no. 8, pp. 773–785, 2003. View at Publisher · View at Google Scholar · View at Scopus
20. Brent GA. Braveman LE. Zoeller RT. Thyroid health and environment.Thyroid 2007:17:807_809.
21. Strieder TG[1], Prummel MF, Tijssen JG, Endert E, Wiersinga WM Risk factors for and prevalence of thyroid

disorders in a cross-sectional study among healthy female relatives of patients with autoimmune thyroid disease ClinEndocrinol (Oxf). 2003 Sep;59(3):396-401

22. Dietary supplement fact sheet: iodine. Office of Dietary Supplements website.http://ods.od.nih.gov/factsheets/Iodine-QuickFacts. Reviewed June 24, 2011.Accessed January 17, 2012.

23.Wreesmann VB, Ghossein RA, Hezel M, et al. Follicular variant of papillary thyroid carcinoma: genome-wide appraisal of a controversial entity. Genes Chromosomes Cancer. 2004 Aug. 40(4):355-64. [Medline].

24. Guideline] NCCN Clinical Practice Guidelines in Oncology: Thyroid Carcinoma Version 2.2014. National Comprehensive Cancer Network. Available at http://www.nccn.org/professionals/physician_gls/PDF/thyroid.pdf. Accessed: September 10, 2014

25. Schlumberger M, Carlomagno F, Baudin E, Bidart JM, Santoro M (2008). "New therapeutic approaches to treat medullary thyroid carcinoma". Nat Clin Pract Endocrinol Metab **4** (1): 22–32. doi:10.1038/ncpendmet0717. PMID 18084343.

26. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A, et al. AJCC Cancer Staging Manual. 7th ed. New York, NY: Springer-Verlag; 2010.

27. NCCN Clinical Practice Guidelines in Oncology: Thyroid Carcinoma. V.2.2013. Available at http://www.nccn.org/professionals/physician_gls/pdf/thyroid.pdf. Accessed: September 5, 2013.

28. El Mezni F, Saibou A, Kooli H, Zermani R, Ferjaoui M, Ben Jilani S. Value of cytology in the diagnosis of thyroid nodules (93 cases) Tunis Med. 2002;80:312–6

29. Thomas JO, Adeyi OA, Nwachokor FN, Olu-Eddo AO. Fine needle aspiration cytology in the management of thyroid enlargement: Ibadan experience. East Afr Med J. 1998;75:657–9.

30. Mitsutake N, Iwao A, Nagai K, Namba H, Ohtsuru A, Saenko V, et al. Characterization of side population in thyroid cancer cell lines: cancer stem-like cells are enriched partly but not exclusively. Endocrinology (2007) 148(4):1797–80310.1210/en.2006-1553

31. Lan L, Luo Y, Cui D, Shi BY, Deng W, Huo LL, et al. Epithelial-mesenchymal transition triggers cancer stem cell generation in human thyroid cancer cells. Int J Oncol (2013) 43(1):113–2010.3892/ijo.2013.1913

32. Takano T. Fetal cell carcinogenesis of the thyroid: a modified theory based on recent evidence [My Opinion]. Endocr J (2014).10.1507/endocrj.EJ13-0517.

33. Park CH, Bergsagel DE, McCulloch EA. Mouse myeloma tumor stem cells: a primary cell culture assay. Journal of National Cancer Institute. 1971;46:411–422

34. Taipale J, Beachy PA. The Hedgehog and Wnt signalling pathways in cancer. Nature. 2001;411:349–354.

35. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. PNAS. 2003;100:3983–3988. [PMC free article] [PubMed]

36. Takano T, Amino N. Fetal cell carcinogenesis: a new hypothesis for better understanding of thyroid carcinoma. Thyroid. 2005;15:432–438.

37.Takeda J, Seino S, Bell GI (Sep 1992). "Human Oct3 gene family: cDNA sequences, alternative splicing, gene organization, chromosomal location, and expression at low levels in adult tissues". Nucleic Acids Research20 (17): 4613–20. doi:10.1093/nar/20.17.4613. PMC 334192.PMID 1408763

38. Young Lab- Core Transcriptional Regulatory Circuitry in Human Embryonic Stem Cells at MIT

39. K. Takahashi, K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda, and S. Yamanaka, Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131 (2007) 861-72.

40 P. Sotomayor, A. Godoy, G.J. Smith, and W.J. Huss, Oct4A is expressed by a subpopulation of prostate neuroendocrine cells. Prostate (2008).
41 K. Hochedlinger, Y. Yamada, C. Beard, and R. Jaenisch, Ectopic expression of Oct-4.
42. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr, accessed December 2013.
43. Hofman MS (2013). "Thyroid nodules: Time to stop over-reporting normal findings and update consensus guidelines". BMJ 347: f5742. doi:10.1136/bmj.f5742. PMID 24068719.
44. "Thyroid Cancer". MedicineNet.com. Retrieved 26 October 2011.
45. Numbers from EUROCARE, from Page 10 in: F. Grünwald; Biersack, H. J.; Grںunwald, F. (2005). Thyroid cancer. Berlin: Springer. ISBN 3-540-22309-6.
46. "FDA approves new treatment for rare form of thyroid cancer". Retrieved 7 April 2011.
47. Carina V, Zito G, Pizzolanti G, Richiusa P, Criscimanna A, Rodolico V, Tomasello L, Pitrone M, Arancio W, Giordano C. Multiple pluripotent stem cell markers in human anaplastic thyroid cancer: the putative upstream role of SOX2Thyroid. 2013 Jul;23(7):829-37. doi: 10.1089/thy.2012.0372. Epub 2013 Jun 21.
48. J. D. Webster1,2,V. Yuzbasiyan-Gurkan1,3,4, J. E. Trosko1,5, C.-C. Chang1,5M. Kiupel1,2Expression of the Embryonic Transcription Factor Oct4 in Canine Neoplasms: A Potential Marker for Stem Cell Subpopulations in Neoplasia16858970[PubMed - indexed for MEDLINE]
49.Wang H[1], Wang JSignificance of Oct-4's expression in thyroid neoplasmLin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi. 2010 Aug;24(15):682-5.

50.Niwa H, Miyazaki J, Smith AG: Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nat Gen 24:372–376, 2000

51.Rosner MH, Vigano MA, Ozato K, Timmons PM, Poirier F, Rigby PW, Staudt LM: A POU-domain transcription factor in early stem cells and germ cells of the mammalian embryo. Nature 345:686–692, 1990

52.Trosko JE: The role of stem cells and gap junctional intercellular communication in carcinogenesis. J Biochem Mol Biol 36:43–48, 2003

53.Potter VR: Phenotypic diversity in experimental hepatomas: the concept of partially blocked ontogeny. Br J Cancer 38:1–23, 1987

54. Cheng L: Establishing a germ cell origin for metastatic tumors using OCT4 immunohistochemistry. Cancer 101:2006–2010, 2004

55. Ezeh UI, Turek PJ, Reijo RA, Clark AT: Human embryonic stem cell genes OCT4, NANOG, STELLAR, and GDF3 are expressed in both seminomas and breast carcinoma. Cancer 104:2255–2265, 2005