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Embryonic stem cell markers expression in cancers

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Abstract:

The transcription factors Oct4 and Sox2 are highly expressed in embryonic stem (ES) cells. In conjunction with Klf4 and c-Myc, their over-expression can induce pluripotency in both mouse and human somatic cells, indicating that these factors are key regulators of the signaling network necessary for ES cell pluripotency. Self-renewal is a hallmark of stem cells and cancer and stemness program could play an important role in cancer.

Therefore we compared the expression of Oct4, Sox2, Klf4 and c-Myc in 40 human tumor types to that of their normal tissue counterparts using publicly available gene expression data, including the Oncomine Cancer Microarray database.

We found significant overexpression of at least 1/4 pluripotency factors Oct4, Sox2, Klf4 or c-Myc in 18 out of the 40 cancer types investigated. Furthermore, within a given tumor category these genes are associated with tumor progression or bad prognosis. A key goal in cancer research is to identify the mechanism by which cancer stem cells arise and self-renew. The overexpression of Oct3/4, Sox2, Klf4 and/or c-Myc could contribute to the pathologic self-renewal characteristics of cancer stem cells.

Introduction

Oct3/4, Sox2, Klf4, c-Myc are 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[1; 2].

Cancer stem cells refer to a subset of tumor cells that has the ability to self-renew and generate tumor heterogeneity [3; 4; 5].

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[6]. Oct4A expressing cells are present in human benign and malignant prostate glands and the frequency of Oct4A expressing cells increases in prostate cancers[6]. A subpopulation of the Oct4A expressing cells co-expressed Sox2, an ES cell marker[6]. In the intestine, Oct4 expression causes dysplasia by inhibiting cellular differentiation in a manner similar to that in the ES cells[7].

In the mouse nervous system, Sox2 is expressed in neural stem cells, in early precursors and in few mature neurons, and is essential to maintain the cell proliferative potential[8; 9]. Sox2 is also expressed in several malignant tissues[10; 11; 12; 13]. These data and the known role of Sox2 in development and cell differentiation suggest that this transcription factor may be relevant to the aberrant growth of tumor cells.

Myc has a gene activation function, a finding consistent with Myc ability to recruit multiple coactivator complexes[14; 15]. The ability of Myc to bind to a staggeringly large number of genomic loci has also been demonstrated in ES cells[16] and is likely to

underlie its activity as part of the “magic quartet” of transcription factors that can reprogram somatic cells to pluripotency [1; 17; 18; 19]. High levels of Myc may block cell differentiation and enhance self-renewal of committed and differentiated cells[20]. During tumor progression, Myc would promote the formation of cancer-initiating cells that retain developmental plasticity. Expression of Myc in hematopoietic stem cells leads to the rapid formation of pre B-cell lymphomas if apoptosis is blocked by coexpression of Bcl-2. Cells derived from these lymphomas can be differentiated into either B-lymphocytes or macrophages in vitro[21]. Similarly, cells derived from liver tumors generated using an inducible Myc transgene differentiate into hepatocytes and biliary cells that go on to form bile ducts when the transgene is turned off [22]. A key function of Myc during normal development is to drive expansion of transit amplifying cells. Due to this unique combination of properties, oncogenic deregulation of Myc expression generates cells possessing a tumor phenotype that has no counterpart during normal development[20].

Kruppel-like factor 4 (Klf4) is a transcription factor expressed in a wide variety of tissues in humans and is important for many different physiological processes, including development, differentiation, and maintenance of normal tissue homeostasis[23]. Klf4 is a transcription factor that can either activate or repress transcription, depending on the target gene. Klf4 can function as an oncogene or a tumor suppressor depending on the type of cancer involved[23]. Klf4 is an anti-proliferative factor in differentiated epithelia, and acts as a tumor suppressor in gastrointestinal cancers[24; 25]. However, Klf4 might also act as an oncogene [26]. It is overexpressed in laryngeal squamous cell carcinoma as an early event in its progression[27]. Klf4 expression is increased in ductal carcinoma of the breast[28] and is associated with an aggressive phenotype and poorer prognosis[29]. In the skin, overexpression of Klf4 results in hyperplasia and dysplasia,

eventually leading to squamous cell carcinoma[30; 31].

Recently, Ben-Porath et al. identified a subset of ES-cell associated transcription regulators that are highly expressed in poorly differentiated tumors revealing a link between genes associated with ES cell identity and the histopathology of tumors[32].

Cancers arise from a cancer stem cell, able of self-renewal and forming tumor heterogeneity. The frequency of cancer stem cells is a matter of debate, depending of the technique used to detect it and the cancer type[5; 33]. Given the critical role of Oct3/4, Sox2, Klf4 and c-Myc to confer on adult cells self-renewal and pluripotency and their role in cancer, we have reviewed here the expression of Oct3/4, Sox2, Klf4 and c-Myc in various cancers, in comparison with their normal counterparts and in association with cancer staging.

Methods

Databases

We used Oncomine cancer microarray database (<http://www.oncomine.org>)[34], Amazonia database (<http://amazonia.montp.inserm.fr/>)[35] and RAGE database (<http://rage.montp.inserm.fr.gate2.inist.fr>)[36] to study gene expression of Oct3/4, Sox2, Klf4 and c-Myc in 40 human tumor types and their normal tissue counterparts as indicated in Table 1. In order to compare the gene expression in a tumor type to its normal counterpart, gene expression data from a same study, performed with the same methodology, were used. The gene expression data were log transformed, median centered per array, and the standard deviation was normalized to one per array[34]. A gene was considered as overexpressed when its mean value in tumor samples was

significantly higher to its mean value in the normal tissue counterpart using a t test ($P \leq .05$).

Statistical analysis

Statistical comparisons were done with Mann Whitney or student t-test.

Results and discussion

We investigated the expression of Oct3/4, Sox2, Klf4 and c-Myc in cancer using publicly available gene expression data. The 40 tumor types investigated corresponded to 4 hematological malignancies and 36 solid tumors (Table 1).

Oct4 is overexpressed in solid and hematological malignancies

We found *Oct4* overexpression in 1/4 hematological cancers and 10/36 solid tumors (Table 1). Overexpression of *Oct4* was found in CLL; oligodendroglioma, glioblastoma multiforme and astrocytoma; in bladder carcinoma; in primary colon cancer; in lung adenocarcinoma; in prostate cancer; in testis tumor; in ovarian carcinoma; in pancreatic cancer and in clear cell carcinoma compared to their normal counterpart tissues (Supplementary Figure S1).

Sox2 is overexpressed in solid tumors

Sox2 was significantly overexpressed in 7/36 solid tumors (Table 1). Overexpression of Sox2 was found in brain cancers; in hepatocellular carcinoma; in bladder carcinoma; in primary colon cancer; in lung adenocarcinoma; in prostate cancer and in seminoma compared to their normal counterpart tissues (Supplementary Figure S2).

KLF4 is overexpressed in all hematological malignancies and in some solid tumors

Klf4 was significantly overexpressed in in 1/4 hematological cancers and 7/36 solid tumors (Table 1): in acute lymphoblastic leukemia; in hairy cell leukemia; in multiple myeloma; in brain tumors; in prostate cancer; in yolk sac tumor compared to their normal counterpart tissues (Supplementary Figure S3).

c-Myc is overexpressed in solid and hematological malignancies

We found *c-Myc* overexpression in all hematological cancers and in 10/36 solid tumors (Table 1). Overexpression of *c-Myc* was found in leukemia; in lymphoma; in smoldering myeloma and in multiple myeloma; in brain tumors; in head and neck cancer; in primary colon cancer compared to normal adjacent mucosa; in breast carcinoma compared to normal breast; in lung adenocarcinoma; in tongue squamous cell carcinoma; in pancreatic cancer; in prostate cancer; in seminoma; in clear cell carcinoma and in salivary-gland tumors compared to their normal counterpart tissues (Supplementary Figure S4).

Correlations between Oct4, Sox2, Klf4 and c-Myc expression in tumor cells

Oct4, Sox2, Klf4 and Myc are all co-overexpressed in 2/40 tumors: *i.e.* in prostate and brain cancers compared to normal counterparts (Table 1). 3 of these 4 factors were co-overexpressed in leukemia, lung tumors and testis cancer compared to their normal counterparts (Table 1). 2 factors were co-overexpressed in 5/40 tumor types: *i.e.* multiple myeloma, colon, bladder, pancreas and kidney. At least 1 out of the 4 pluripotency factors were overexpressed in 3/4 hematological cancers and in 15/40 solid tumors.

Link between Oct4, Sox2, Klf4 and c-Myc expression and tumor grade

Thus, we sought to find out whether *Oct4*, *Sox2*, *Klf4* and *Myc* expression could be associated with tumor progression and prognosis.

Oct4 was significantly overexpressed in glioma grade IV compared to grade II and III; in poorly differentiated breast carcinoma compared to well differentiated tumors from patients; in head and neck cancers grade IV compared to grade I and in metastatic sarcoma compared to non metastatic sarcoma (Table 2 and Supplementary Figure S5).

Sox2 is overexpressed in bladder carcinoma grade III compared to grade I; in glioma grade IV compared to grade II and III; in head and neck cancers grade III and IV compared to grade I and II; in breast tumors grade III compared to grade I in 8 independent studies; in colorectal carcinoma T stage 4 compared to T stages Tis and T1; in cervical carcinoma stage IV compared to stage I; in endometrial carcinoma stage IV compared to stage I and T stage 4 compared to T stage 1; in metastatic sarcoma compared to non metastatic sarcoma; in metastatic papillary renal cell carcinoma compared to non metastatic papillary renal cell carcinoma and in thyroid gland carcinoma stage IV compared to stage I (Table 2 and Supplementary Figure S6).

Klf4 is overexpressed in metastatic ovarian carcinoma compared to non metastatic ovarian carcinoma; in prostate carcinoma stage III compared to stage II and in primary melanoma tumors N stage 1 compared to N stage 0 (Table 2 and Supplementary Figure S7).

C-Myc is overexpressed in poorly differentiated lung adenocarcinoma compared to well differentiated lung carcinoma; in ovarian carcinoma grade IV compared to grade I; in poorly differentiated pancreatic ductal adenocarcinoma compared to well differentiated pancreatic ductal adenocarcinoma; in breast carcinoma grade III compared to grade I; in

breast ductal carcinoma stage N1 compared to stage N0; in mantle cell lymphoma grade IV compared to grade I; in metastatic sarcoma compared to primary sarcoma and in melanoma stage N1 compared to stage N0 (Table 2 and Supplementary Figure S8).

Sox2 has been found expressed in a variable percentage of cells in several malignant tissues[10; 11; 12; 13] and is essential to maintain the cell proliferative potential of neural stem cells[8; 9]. Furthermore Sox2 is overexpressed in patients with ovarian carcinoma dead at 5 years compared to patients alive at 5 years ($P = .001$)[37] and in patients with melanoma metastasis tumor dead at 3 years compared to patients alive at 3 years ($P = .007$)[38] (Table 3 and Figure 1).

C-Myc is overexpressed in patients with non-small cell lung cancer dead at 5 years compared to patients alive at 5 years ($P = .007$)[39]; in patients with glioma dead at 3 years compared to patients alive at 3 years ($P = .008$)[40]; in patients with breast carcinoma dead at 5 years compared to patients alive at 5 years ($P = .007$)[41]; in patients with diffuse large B-cell lymphoma dead at 5 years compared to patients alive at 5 years ($P = .006$ and $P = .007$)[42; 43]; in patients with Burkitt's lymphoma dead at 5 years compared to patients alive at 5 years ($P = .006$)[44] and in 1 year relapsing multiple myeloma patients compared to patients with no relapse at 1 year ($P = .001$)[45] (Table 3 and Figure 1). Oct4 and Klf4 expressions were not associated with prognostic in the 40 tumor types investigated (Table 3).

Conclusions:

A key goal in cancer research is to identify the mechanism by which cancer stem cells arise and self-renew. We have shown here that at least one gene coding for 1/4 pluripotency factors - Oct4, Sox2, Klf4, and c-Myc - is overexpressed in 18/40 cancer types investigated. In addition, for a given tumour, the expression of these genes is

associated with tumor progression or bad prognosis. The activation of an ES cell-like transcriptional program in differentiated adult cells may induce pathologic self-renewal characteristics of cancer stem cells. In particular, it was recently demonstrated that c-Myc activates an embryonic stem cell like program in epithelial cells leading to epithelial tumor initiating cells[46]. Cancer stem cells are so named because they possess qualities reminiscent of normal tissue stem cells including self-renewal, prolonged survival, and the ability to give rise to cells with more differentiated characteristics. Effort is now focused on identifying cancer stem cells in various malignancies, and defining the cells of origin. This review of publicly available data emphasizes the cancer types associated with Oct3/4, Sox2, Klf4 and/or c-Myc overexpression and may encourage further studies to address the role of these pluripotent transcription factors in these cancers.

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Figure legends

Figure 1: Association of Sox2 and c-Myc expression with prognosis

A. c-Myc expression in multiple myeloma relapsing patients and patients with no relapse[45], in alive patients with and dead patients with breast carcinoma,[41] , in MM patients with no relapse, in MM patients with relapse[45], in alive and dead patients with diffuse large B cell lymphoma[42; 43], in alive and dead patients with Burkitt's lymphoma[44], in alive and dead patients with lung cancer[39], in alive and dead patients with brain cancer[40].

B. Sox2 expression in alive and dead patients with ovarian carcinoma [37] and in alive and dead patients with melanoma metastasis tumor[38]. Patients with multiple myeloma without Sox2 expression (Affymetrix call) have a better event free survival (EFS) and overall survival (OAS) than patients expressing Sox2.

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Table 1

Tissue	Oct4	Sox2	KLF4	c-MYC
Lymphoma	no	no	no	yes
Leukemia	yes	no	yes	yes
Myeloma	no	no	yes	yes
Adrenal	no	no	no	no
Bladder	yes	yes	no	no
Blood	no	no	no	no
Brain	yes	yes	yes	yes
Breast	no	no	no	yes
Cervix	no	no	no	no
Chondrosarcoma	no	no	no	no
Colon	no	yes	no	yes
Endocrine	no	no	no	no
Endometrium	no	no	no	no
Esophagus	no	no	no	no
Gastric	no	no	no	no
Head-Neck	no	no	no	yes
Liver	no	yes	no	no
Lung	yes	yes	no	yes
Melanoma	no	no	no	no
Mesothelioma	no	no	no	no
Multi-cancer	no	yes	no	no
Muscle	no	no	no	no
Neuroblastoma	no	no	no	no
Oral	no	no	no	no
Others	no	no	no	no
Ovarian	yes	no	no	no
Pancreas	yes	no	no	yes
Parathyroid	no	no	no	no
Prostate	yes	yes	yes	yes
Rectum	no	no	no	no
Renal	yes	no	no	yes
Salivary-gland	no	no	no	yes
Sarcoma	no	no	no	no
Seminoma	yes	yes	no	yes
Skin	no	no	no	no
Testis	yes	yes	yes	no
Thyroid	no	no	no	no
Uterus	no	no	no	no

Table 1

Over-expression of *SOX2*, *OCT4*, *KLF4* and *c-MYC* in human tumor types to that of their normal tissue counterparts using publicly available gene expression data, including the Oncomine Cancer Microarray database.

Table 2

cancer type	sox2	Oct4	Klf4	c-Myc
Bladder	+			
Brain	+	+	+	
Breast	+	+		+
Cervix	+			
Colon	+			
Endometrium	+			
Head-Neck	+	+		
Lung				+
Lymphoma				+
Melanoma			+	+
Ovarian			+	+
Pancreas				+
Prostate			+	
Renal	+			
Sarcoma	+	+		+
Thyroid	+			

Table 2

Association sox2, oct4, Klf4 and c-Myc with tumor grade

Table 3

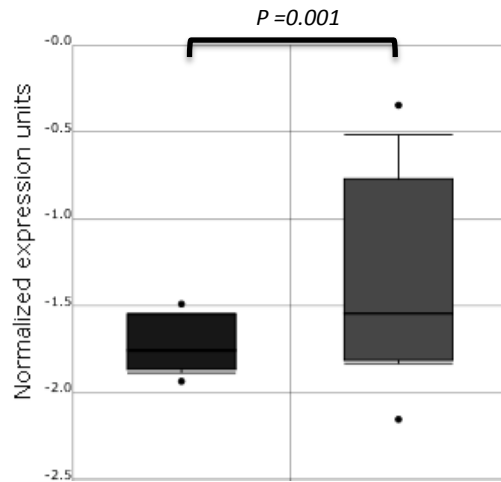
cancer type	sox2	Oct4	Klf4	c-Myc
Bladder				
Brain				+
Breast				+
Colon				
Head-Neck				
Leukemia				
Liver				
Lung				+
Lymphoma				+
Melanoma	+			
Myeloma	+			+
Ovarian	+			
Prostate				
Renal				

Table 3

Association of sox2, Oct4, Klf4 and c-Myc with prognosis in cancer.

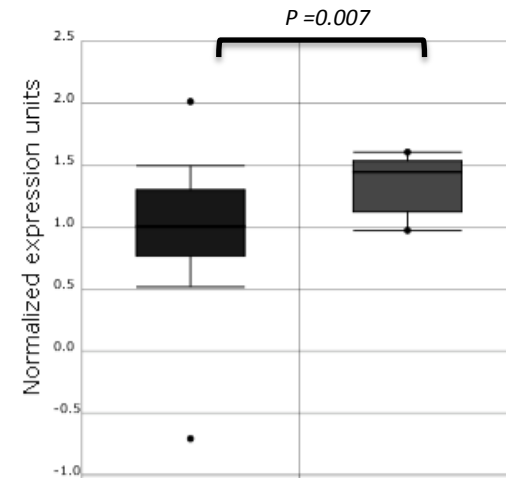
Figure 1 A : Association of c-Myc expression with prognosis

Multiple Myeloma - Relapse Status - 1 Year



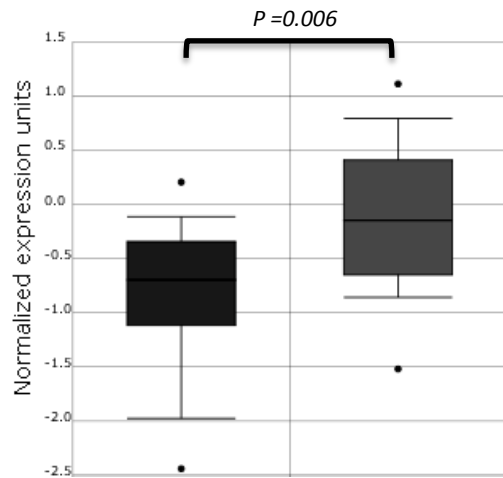
No Relapse (11) Relapse (26)
Carrasco et al. Cancer Cell 2006

Breast Carcinoma - Overall Survival - 5 Years

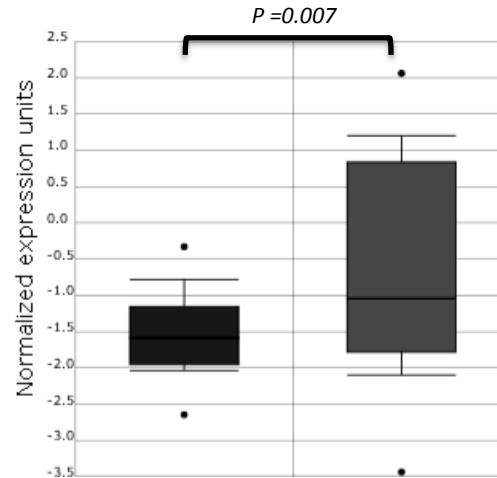


Alive (166) Dead (8)
Desmedt et al. Clin Cancer Res 2007

Diffuse Large B-Cell Lymphoma - Survival - 5 Years

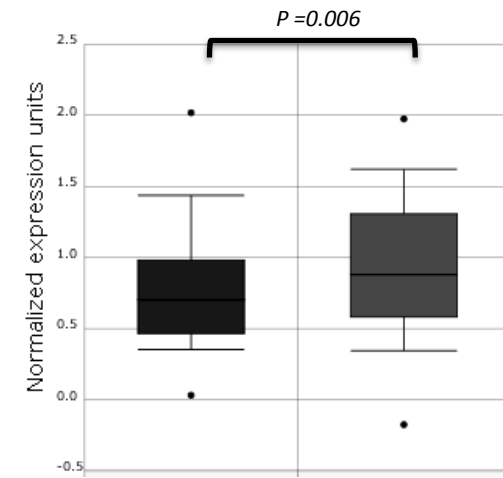


Alive (15) Dead (26)
Shipp et al. Nat Med 2002



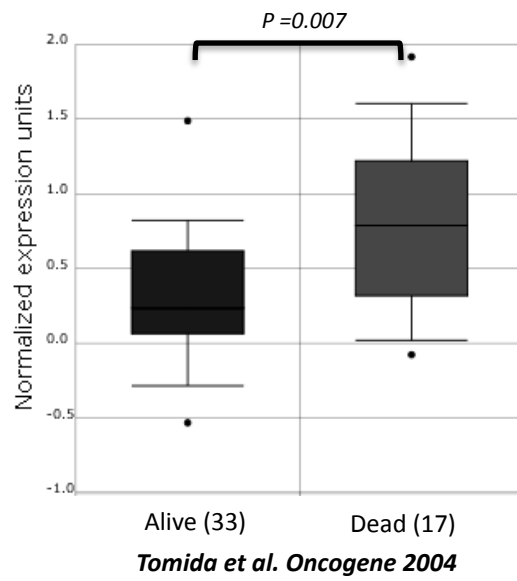
Alive (23) Dead (31)
Alizadeh et al. Nature 2000

Burkitt's Lymphoma - Survival - 5 Years



Alive (87) Dead (137)
Dave et al. N Engl J Med 2006

Non-Small Cell Lung Cancer - Survival - 5 Years



Brain - Survival - 3 Years

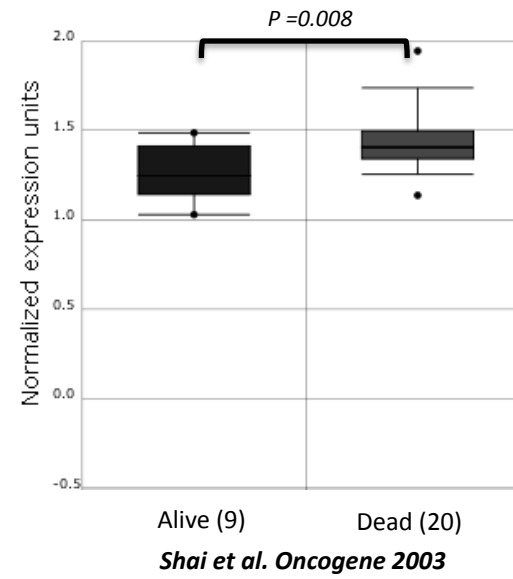
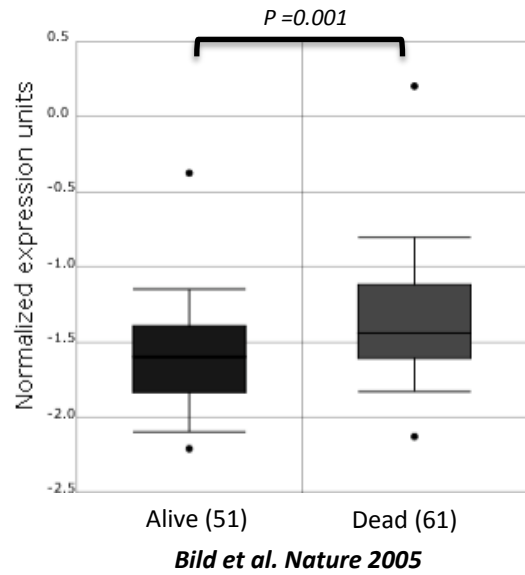
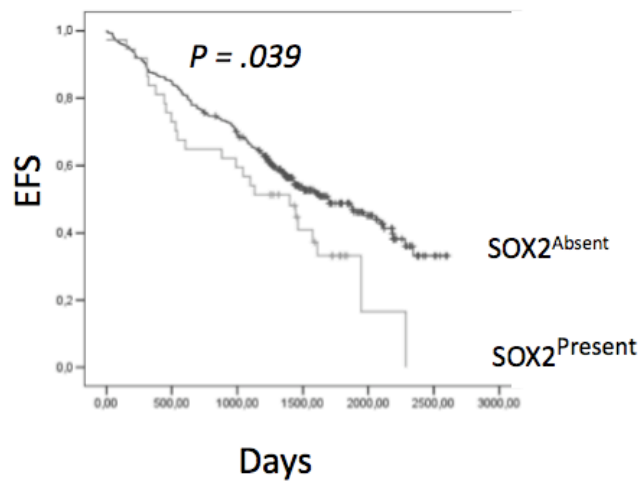
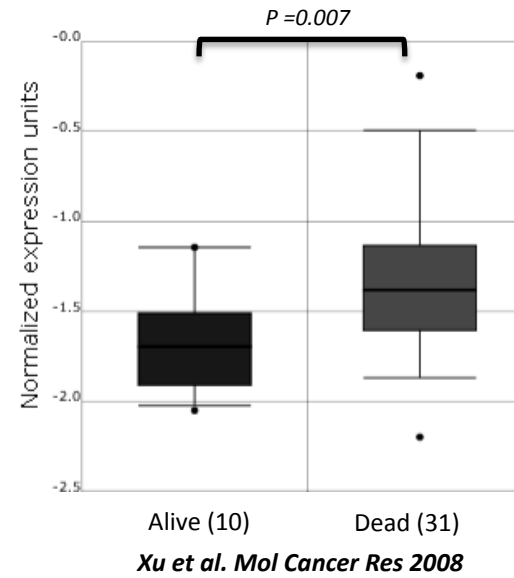


Figure 1 B : Association of Sox2 expression with prognosis

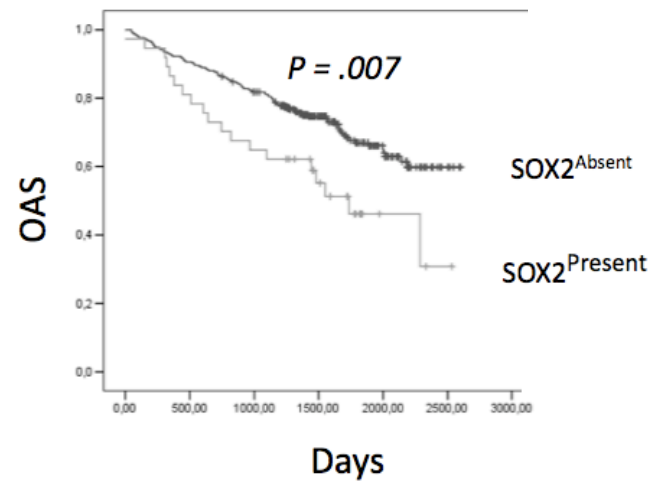
Ovarian Carcinoma - Survival - 5 Years



Melanoma Metastasis Tumor - Survival - 3 Years



SOX2	Median
SOX2 ^{Absent}	1698 days
SOX2 ^{Present}	1399 days



SOX2	Median
SOX2 ^{Absent}	1466 days
SOX2 ^{Present}	1611 days