Gene Ther Mol Biol Vol 15, 1-7, 2013

The significant aberrant expression of *FOXC1* as a high specific and sensitive potential biomarker in gastric adenocarcinoma tumor tissues

Research Article

Mohammadreza Hajjari[1], Mehrdad Behmanesh[1] and Mehdi Zeinoddini[2]

[1] Department of Genetics, School of Biological Sciences, Tarbiat Modares University, Tehran, Iran.

[2] Biotechnology Research Center, Mallek Ashtar University of Technology, Tehran, Iran.

***Correspondence:** Mehrdad Behmanesh, Department of Genetics, School of Biological Sciences, Tarbiat Modares University, Tehran, Iran, P.O. Box 14115-154, Tel +98-21-8288-4451, Fax: +98-21-8288-4717. Email: behmanesh@modares.ac.ir

Keywords: FOX transcription factors, EMT, Gastric Cancer, Quantitative Real time-PCR

Received: 25 April 2013; Revised: 31 May 2013

Accepted: 1 June 2013; electronically published: 2 June 2013

Summary

FOX transcription factors regulate a wide array of biological processes including development, differentiation, and invasion. It seems that some FOX proteins are involved in epithelial-to-mesenchymal transition (EMT), which is involved in the progression of cancer. However, the role of *FOXC1*, one of the members of FOX proteins, remains unknown in the progression of gastric adenocarcinoma. Here, by quantitative Real time PCR, we report the significant up-regulation of *FOXC1* in gastric tumors compared with normal tissues. Also, because it was previously reported that *FOXC1* expression is correlated with *SUZ12* in breast cancer tissues, we wondered if this correlation exists in gastric cancer tissues. Concerning the role of *SUZ12*, one of the components of Polycomb repressive Complex 2, unraveling the relation between *FOXC1* and *SUZ12* may help us to decipher the mechanisms of gastric adenocarcinoma. In the current study, we demonstrate that *FOXC1* expression can be considered as a good putative biomarker for gastric adenocarcinoma tissues by ROC curve analysis. Based on our results, *FOXC1* may potentially be useful as a biomarker or molecular target in treatment or prevention of gastric cancer.

I. Introduction

Gastric cancer is one of the most common types of cancer in the world, and approximately one million patients are diagnosed each year (Shah MA and Kelsen DP, 2010). Although several environmental factors, such as *Helicobacter pylori* infection, excessive intake of salt, as well as the low intake of vegetables and fruit, have been linked with gastric carcinogenesis, the molecular mechanisms underlying gastric carcinogenesis are poorly understood. In recent years, many studies have focused on finding genes which are chiefly involved in the progression of gastric carcinogenesis (Cui J, et al., 2011; Hajjari M, et al., 2013; Peek Jr RM and Blaser MJ, 2002; Yuasa Y). Disclosing the role of these genes may help us to understand the mechanisms of gastric cancer progression precisely.

FOXC1 is a member of the superclass of Forkhead transcription factors, which have different roles in various biological processes. Fox proteins have a characteristic forkhead or winged-helix (WH) DNA-binding domain, and are required for a variety of functions such as the maintenance of differentiated cell states, tissue-specific gene expression and embryogenesis (Hannenhalli S and

Kaestner KH, 2009; Kaufmann E and Knochel W, 1996; Kidson SH, et al., 1999). *FOXC1* gene is located on human chromosome 6p25 (Nishimura, et al., 1998) and its transcript has been detected in multiple human organs using Northern blot analysis (Nishimura, et al., 1998; Pierrou, et al., 1994). Systemic analysis of phenotypic change in *FOXC1*-null mice revealed that *FOXC1* expression is important for developmental processes (Mears AJ, et al., 1998).

There are some reports showing the potential role of *FOXC1* in the initiation and the progression of different cancers. Recently, Ray et al. reported that *FOXC1* overexpression is a consistent feature of early invasive and basal-like breast cancer (Ray PS, et al., 2010). Muggerud et al. reported that *FOXC1* knockdown suppresses cell proliferation, migration and invasion in breast cancer cells (Muggerud AA, et al., 2010). Furthermore, in some studies, *FOXC1* has been suggested as an inducer of epithelial to mesenchymal transition (EMT) (Bloushtain-Qimron N, et al., 2008). EMT process has been described in epithelial carcinogenesis (Bloushtain-Qimron N, et al., 2008; Polyak K and Weinberg RA, 2009).

The potential role of *FOXC1* in tumorigenesis and epithelial to mesenchymal transition prompted us to investigate the possible role of this gene in the initiation and/or progression of gastric adenocarcinoma, one of the most important epithelia originating cancer. This study was done to examine the expression levels of *FOXC1* in gastric cancer specimens and normal adjacent tissues derived from the same patients. We assessed the correlation of *FOXC1* expression with clinopathological parameters and demonstrated, for the first time, that *FOXC1* is aberrantly expressed in gastric adenocarcinoma tissues.

Since it was previously reported that the expression of *FOXC1* is negatively correlated with *SUZ12* expression in breast cancer (Du J, et al., 2012), we tried to find if this correlation exists in gastric adenocarcinoma tissues. This may help us to decipher the mechanism of *FOXC1* function in gastric adenocarcinoma. *SUZ12* is one of the components of Polycomb repressive complex 2 and can downregulate some genes involved in cancer progression by histone modification (Kirmizis A, et al., 2004). Finding any coopereative expression with this gene might reveal some pathways involved in cancer progression. We examined *SUZ12* mRNA expression level in specimens and analyzed its correlation with *FOXC1* mRNA level.

II. Materials and Methods

A. Tissue Samples

A total of 62 gastric tissue samples (31tumor and 31adjacent noncancerous gastric epithelia specimens) were obtained from Iran National Tumor Bank (Tehran, Iran). The patients consisted of 18 males and 13 females with a mean age of 64± 20 years old. Informed consent was obtained from all patients before sampling. Clinopathological information was obtained from medical charts and two independent pathologists examined the histopathological parameters. Fresh gastric mucosa specimens were frozen immediately in time of resection. The Medical Ethics Committee of Tarbiat Modares University approved the experimental design and procedure.

B. RNA Extraction and cDNA synthesis

Total RNA was extracted using RNX™-plus solution (Cinnagen, Iran) according to the manufacturer's instructions. RNA was treated with DNase I (Sigma) at 37ºC for 30 min to omit any genomic DNA contamination. The purity and integrity of extracted RNAs were measured by spectrophotometer at 260 and 280 nm and running on agarose gel electrophoresis, respectively. cDNA was generated by reverse transcription of 3μg of total RNA using RevertAid™ Reverse Transcriptase (Fermentas, Canada) with oligo (dT)18 and random hexamer primers (MWG, Germany) in a total 20 μl reaction mixture, according to the manufacturer's instructions.

C. Real-time polymerase chain reaction (PCR) analysis

Expression analysis was done by real-time quantitative RT-PCR technique with an ABI 7500 sequence detection system (Applied Biosystems, USA) and 10 ng cDNA in a total volume of 20 μl. The reaction was carried out using 10 μl of SYBR Green I master mix (Takara, Shiga, Japan) and 200 nM of sense and antisense primers, according to the manufacturer's instructions.

All appropriate primers were designed and their sequences are shown in Table 2.

The PCR reaction was performed as follows: an initial denaturation at 95ºC for 5 min, followed by 40 cycles of denaturation at 95ºC for 10 s and annealing/extension at 60 ºC for 30 s. Melting curve analysis was consistently performed at the end of the reactions to check for primer–dimer artifacts and contamination. In addition, in all experiments, appropriate negative controls containing no template were subjected to the same procedure to exclude or detect any possible contamination.

The real-time RT-PCR allows, by means of fluorescence emission, the identification of the cycling point when PCR product is detectable. Data are acquired as threshold cycle (Ct) value. The Ct value inversely correlates with the starting quantity of target mRNA for each sample. In our study, measurements were performed at least in duplicate and controls were included in which the reaction mixture contained no cDNA. As the internal control to normalize mRNA levels, amplification of the human glyceraldehyde 3-phosphate dehydrogenase gene (*GAPDH*) was used for quantitative PCR in the analysis of *FOXC1* and *SUZ*12 gene expression in the samples. Because the amplification efficiencies of target genes and the internal control were equal, the relative changes of target genes expression in tumor cells compared with normal gastric epithelium (ΔCT calibrator value) was calculated using the equation $2^{-\Delta\Delta Ct}$ (Livak, et al., 2001).

D. Statistical Analysis

Statistical analyses were performed using SPSS (SPSS Inc., Chicago, IL, USA) and GraphPad softwares. The

comparison of mRNA levels between normal and tumor tissues of patients was done with paired Student's t test.

The clinopathological variables of differentiation grade, lymph node metastasis, and invasion depth were used in this study. Regarding the clinopathological features, the obtained data were analyzed by statistical modeling, and the results from correlation analyses were expressed by Pearson's correlation coefficient. The Receiver Operating Characteristic (ROC) curve was used to analyze the specificity and sensitivity of the difference of the expression level of *FOXC1* as a potential biomarker. The area under the ROC curve (AUROC) was used to represent an overall summary of its diagnostic accuracy. A P-value ≤ 0.05 was considered significant and data were shown as mean ± standard deviation (SD).

III. Results:

A. Clinical Characteristics of the Patients

The patients in this study included eighteen males and thirteen females aged from 44 to 84 years with an average age of 64 years. Among the 31 patients, 21 were diagnosed with non-cardia carcinoma, and 10 patients were diagnosed as cardia stomach cancer type. With regard to the Grade of tumors, 11 cases were categorized as low grade, 9 cases as intermediated grade and 11 cases as high grade according to the result of pathological examinations. The clinopathological data of patients according to the TNM (Tumor, Node, and Metastasis) staging criteria are shown in **Table 1**.)

Clinicopathological characteristics	Cases number
Patients	31
Age	
<64	15
>64	16
Gender	
Male	18
Female	13
Location of Tumor	
Cardia	10
Non-Cardia	21
Invasion depth	
T1-T2	9
T3-T4	22
Lymph Node Metastasis	
Negative	11
Positive	20
Grade of Tumors	
Low Grade	11
Intermediate Grade	9
High Grade	11

Table 1: Clinicopathological characteristics of the patients with gastric adenocarcinoma

B. Gene expression levels in normal gastric and adenocarcinoma tissues

Comparison of *FOXC1* gene expression between gastric tumors and their adjacent normal tissues showed that *FOXC1* gene expression in tumors was significantly higher than that in their adjacent normal tissues (Change fold: 3.81, p-value: 0.015). The result is shown in **Figure 1**.

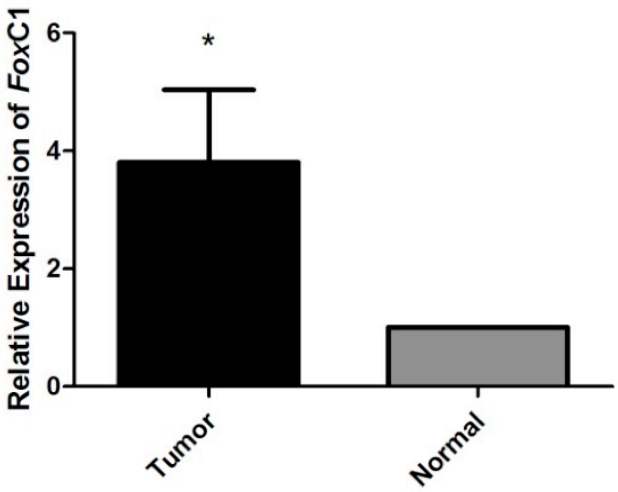

Figure 1: The expression level of *FOXC1* is up-regulated significantly in gastric tumor specimens in comparison to the normal margin respective tissues (P< 0.05). Each real-time PCR examination was carried out in duplicate. The gene expression values were normalized relative to the level of *GAPDH* expression in the respective tissues. The relative change of mRNA expression was calculated using the equation $2^{-\Delta\Delta Ct}$ with Paired t-test (calibrator value=1.0).

C. Expression analysis of *FOXC1* in tumors with different clinicopathologic characteristics

The comparison of FOXC1 gene expression between tumors with different grade, invasion depth or lymph node metastasis did not show any significant differences, as represented in **Table 3 and Figure 2**.

D. Receiver Operating Characteristics (ROC) curve analysis

The up-regulation of FOXC1 showed a highly discriminative receiver operating characteristic (ROC) curve profile, which clearly distinguishes tumors from normal tissues. The area under the ROC curve (AUROC) was 0.76±0.08 (P-Value: 0.001), the 95% confidence interval was 60%–92% and the criterion value (cutoff value) was 1.035. The detection of *FOXC1* expression

yielded 100.00% sensitivity and 74.1% specificity in the diagnosis of gastric cancer **(Figure 3).**

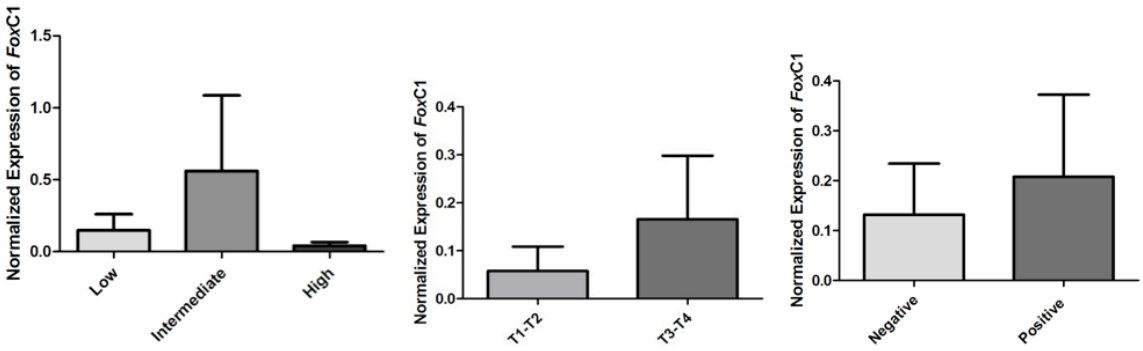

Figure 2: Analysis of genes expression between different grades (a), invasion depth (b) and lymph node metastasis (c) of gastric adenocarcinoma tumors. Although there are some differences between expression levels in tumors, they are not significant. Data are presented as normalized gene expression to an endogenous reference gene (GAPDH) in different tumors.

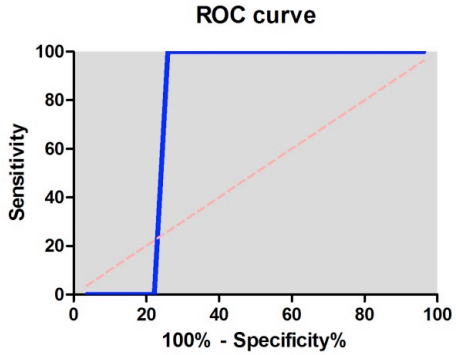

Figure 3: Receiver operating characteristic (ROC) curve for expression change between tumors and normal gastric tissues. Corresponding True Positive Rate (sensitivity) was plotted against the False Positive Rate (1- specificity). AUC shows good distinguish efficiency (p-value: 0.001).

Gene Name (Ref Seq. ID)	Primer Sequence	PCR product Length
FOXC1 (NM_001453.2)	Forward: GCCAGCAGCAGAACTTCCACTC Reverse: TCAAAACTTGCTACAGTCGTAG	167bp
SUZ12 (NM_015355.2)	Forward: GAGAAAGAAGTGATGAAACTCTGG Reverse: TGCATTTCACGGAGCTTGGTAAC	227bp
GAPDH (NM_002046.4)	Forward: CCATGAGAAGTATGACAAC Reverse: GAGTCCTTCCACGATACC	115bp

Table 2: The sequence of primers which were used in this study for gene expression analysis by Real Time PCR

Differentiation Grades	Cases	Normalized Gene expression	*P*-Value
Low	11	0.1479 ± 0.1132	0.3263
Intermediate	9	0.5611 ± 0.5251	
High	11	0.04185 ± 0.02519	

Invasion Depth	Cases	Normalized Gene expression	*P*-Value
T1-T2	9	0.05794 ± 0.05098	0.35
T3-T4	22	0.1659 ± 0.1323	

Lymph Node Metastasis	Cases	Normalized Gene expression	*P*-Value
Negative	11	0.1320 ± 0.1021	0.37
Positive	20	0.2078 ± 0.1647	

Table 3: Normalized expression of genes in different grades, Invasion depth and lymph node metastasis gastric cancer patients. Normalized

amount of gene expression relative to an internal control gene (GAPDH) is shown as mean ± standard deviation

.

E. Correlation between *FOXC1* and *SUZ12* expression levels in tumor tissues

There was not any significant correlation between expression level of *SUZ12* (mean of the expression level: 0.8129±0.1691) and *FOXC1* (mean of the expression level: 0.1345±0.098) in gastric adenocarcinoma tissues (r = -0.1812, p value = 0.1829).

IV. Discussion:

Members of the forkhead box (FOX) transcription factors regulate different biological processes including development and differentiation (Myatt SS and Lam EW, 2007). One of these members is *FOXC1* which was originally identified as a transcription factor playing an important role in regulation of ocular development (Lehmann OJ, et al., 2003). Later, its transcript was detected in multiple human organs and recently, *FOXC1* was recognized as a potential regulator of differentiation (Lehmann OJ, et al., 2003).

Accumulating evidence for alteration of *FOXC1* expression in various types of human cancer suggests that it plays a key role in tumor biology. Du et al. reported that invasion and metastasis of breast cancer cells decreased in response to *FOXC1* over expression (Du J, et al., 2012). Also Zhou et al., showed that the expression of *FOXC1* was lower in ovarian cancer than in normal primary endometrial (Zhou Y, et al., 2002). Interestingly, in some other studies it has been reported that *FOXC1* increases invasion and metastasis in endometrial and breast cancer models (Chung TK, et al., 2012; Ray PS, et al., 2010). Also, over expression of *FOXC1* is observed in other kinds of malignant tumors including hepatocellular carcinoma (Xia L, et al., 2012), prostate cancer (Peraldo-Neia C, et al., 2011), lung cancer (Wei LX, et al., 2013) , and pancreatic cancer (Wang L, et al., 2013). In spite of different studies whose conflictions may be attributed to the context and origination of tumors, the styles of studies, or distinct roles of *FOXC1* in different cancer types, no studies have reported the clinicopathologic significance of *FOXC1* in gastric adenocarcinoma tumors. In this study, we present the first evidence that *FOXC1* expression levels are up-regulated in gastric adenocarcinoma compared to normal gastric tissues. Based on the previous studies on up-regulation of *FOXC1* expressions in cancer progression (Chung TK, et al., 2012; Ray PS, et al., 2010), our data are in concordance with these studies.

Gastric adenocarcinoma is a malignant epithelial tumor, originating from glandular epithelium of the gastric mucosa. Studies have shown that the aberrant activation of Epithelial to Mesenchymal transition process can play a vital role in adult epithelia cancers (Natalwala A, et al., 2008). Since *FOXC1* is involved in EMT progression (Bloushtain-Qimron N, et al., 2008; Xia L, et al., 2012), we first hypothesize that overexpression of this gene in gastric tumors may be attributed to its role in EMT regulating. EMT induces tumor-associated epithelial cells to obtain mesenchymal features, which results in reduced cell-cell contact and increased motility (Thiery JP, 2002). The other proposed potential role in cancer progression for *FOXC1* is interaction with Notch and VEGF pathways (Seo S, et al., 2006; Seo S, et al., 2012) which are deregulated in gastric cancer (Yeh TS, et al., 2009; Zhao R, et al., 2010). Regardless of different proposed potential mechanism for *FOXC1* function, our observation in this study supports the putative role of *FOXC1* as a molecule that may be involved in epithelium originated stomach cancer.

We evaluated the relationship between *FOXC1* expression and various clinicopathological parameters in gastric tumors. Although the numbers of samples were distributed well in different groups, we didn't find any significant association between clinopathological parameters and *FOXC1* expression. However, despite the lack of statistical significance, there was a trend that the expression of *FOXC1* was correlated with grade, invasion, and lymph node metastasis. The results may open vistas for the researchers and the statistical significance may be obtained with large scale follow-up study.
Using Roc curve analysis, the AUROC (Area under curve) identified optimal sensitivity and specificity levels, which can be used to distinguish normal tissues from tumor ones. So, it seems that *FOXC1* expression level may be a good potential biomarker to diagnose gastric adenocarcinoma tissues.

Du et al. claim that Polycomb Repressive Complex 2 functions via targeting *FOXC1* and expression level of *FOXC1* is negatively correlated with PcG genes such as *SUZ12* (Du J, et al., 2012). We wondered how and whether this correlation might be in gastric adenocarcinoma tissues. Finding any correlation between these genes might reveal new insights into their roles in gastric adenocarcinoma progression. In normal adult tissues, expression of PRC2 complex members such as *SUZ12*, *EZH2* (enhancer of zeste homolog 2), and *EED* (Embryonic Ectoderm Development) is very low (Kirmizis A, et al., 2004; Kuzmichev A, et al.), suggesting that the Polycomb repressive complexes may not play a major role in normal differentiated tissues. In contrast, these proteins have been shown to be present at high levels in a variety of human tumors. PRC2 complex promotes gene repression through epigenetic modification of histones. This complex tri-methylates H3K27 in its different target genes (Squazzo SL, et al., 2006). We measured mRNA expression level of *SUZ12* in gastric tumors and found that there is not any significant correlation between *SUZ12* and *FOXC1* mRNA expression levels in tumor tissues. This result may not support the idea that *FOXC1* expression is correlated with

SUZ12 expression in gastric cancer (Du J, et al., 2012). Squazzo et al., in a study using variety of ChIP-chip approaches, identified a large set of SUZ12 target genes in different human cell lines and found that *SUZ12* target promoters are cell type specific (Squazzo SL, et al., 2006). So, targets of SUZ12 may be different in various tissues such as gastric and breast.

In summary, in this study we assessed expression level of *FOXC1* in gastric adenocarcinoma and their normal adjacent tissues. We demonstrated up-regulated expression level of this gene in tumor tissues compared with normal ones and demonstrated its potential to discriminate between tumor and normal gastric tissues. There are some recent reports about the relation between deregulation of forkhead box (FOX) genes and gastric tumorigenesis (Cheng AS, et al., 2013; Jiang C, et al., 2011; Ma GF, et al., 2013) but based on our knowledge, the current study is the first that presents an evidence for deregulation of *FOXC1* in gastric cancer. Accordingly, *FOXC1* can be considered as a potential novel therapeutic target for gastric cancer. Further studies are needed to disclose roles of this gene still further.

Acknowledgments

The authors gratefully acknowledge the contribution of the patients and institutions in this study. The Iranian Stem Cell Network and the Department of Research Affairs of Tarbiat Modares University provided the funding for this work.

References

Bloushtain-Qimron N, Yao J, Snyder EL, Shipitsin M, Campbell LL, Mani SA, HM, Chen H, Ustyansky V, Antosiewicz JE, Argani P, Halushka MK, Thomson JA, Pharoah P, Porgador A, Sukumar S, Parsons R, Richardson AL, Stampfer MR, Gelman RS, Nikolskaya T, Nikolsky Y and Polyak K (2008) Cell type-specific DNA methylation patterns in the human breast. ***Proc Natl Acad Sci U S A*** 105, 14076-14081.

Cheng AS, Li MS, Kang W, Cheng VY, Chou JL, Lau SS, Go MY, Lee CC, Ling TK, Ng EK, Yu J, Huang TH, To KF, Chan MW, Sung JJ and Chan FK (2013) Helicobacter pylori Causes Epigenetic Dysregulation of FOXD3 to Promote Gastric Carcinogenesis. ***Gastroenterology*** 144, 122-133.

Chung TK, Lau TS, Cheung TH, Yim SF, Lo KW, Siu NS, CL, Yu MY, Kwong J, Doran G, Barroilhet LM, Ng AS, Wong RR, Wang VW, Mok SC, Smith DI, Berkowitz RS and Wong YF (2012) Dysregulation of microRNA-204 mediates migration and invasion of endometrial cancer by regulating FOXC1. ***Int J Cancer*** 130, 1036-1045.

Cui J, Li F, Wang G, Fang X, Puett JD and XuY (2011) Gene-Expression Signatures Can Distinguish Gastric Cancer Grades and Stages. ***PLoS ONE*** 6, e17819.

Du J, Li L, Ou Z, Kong C, Zhang Y, Dong Z, Zhu S, Jiang H, Shao Z, Huang B and Lu J (2012) FOXC1, a target of polycomb, inhibits metastasis of breast cancer cells. ***Breast Cancer Res Treat*** 131, 65-73.

Hajjari M, Behmanesh M, Sadeghizadeh M and Zeinoddini M (2013) Junctional adhesion molecules 2 and 3 may potentially be involved in progression of gastric adenocarcinoma tumors. ***Med Oncol*** 30(1),380.

Hannenhalli S and Kaestner KH (2009) The evolution of Fox genes and their role in development and disease. ***Nat RevGenet*** 10, 233-240.

Jiang C, Wang W, Yan W, Zhang Y, Yang J, Zhang S, Zhang C, Zhang W, Han W, Wang J and Zhang YQ (2011) Clinical implications and characteristics of factor forkhead box protein 3 in gastric cancer. ***Exp Ther Med*** 2, 667-673.

Kaufmann E and Knochel W (1996). Five years on the wings of fork head. ***Mech Dev*** 57(1), 3-20.

Kidson SH, Kume T, Deng K, Winfrey V and Hogan BL (1999) The forkhead/winged-helix gene, Mf1, is necessary for the normal development of the cornea and formation of the anterior chamber in the mouse eye. ***Dev Biol*** 211, 306-322.

Kirmizis A, Bartley SM, Kuzmichev A, Margueron R, Reinberg D, Green R and Farnham PJ (2004) Silencing of human Polycomb target genes is associated with methylation of histone H3 lysine 27. ***Genes & Dev*** 18, 1592-1605.

Kuzmichev A, Margueron R, Vaquero A, Preissner TS, Scher M, Kirmizis A, Ouyang X, Brockdorff N, Abate-Shen C, Farnham P and Reinberg D (2005) Composition and histone substrates of Polycomb repressive group complexes change during cellular differentiation. ***Proc. Natl. Acad. Sci*** 102, 1859-1864.

Lehmann OJ, Sowden JC, Carlsson P, Jordan T and Bhattacharya SS (2003) Fox's in development and disease. ***Trends Genet*** 19, 339-344.

Livak KJ, Schmittgen TD (2001) Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2–ΔΔCT Method. ***Methods*** 25,402-408.

Ma GF, Chen SY, Sun ZR, Miao Q, Liu YM, Zeng XQ, Luo TC, Ma LL, Lian JJ and Song DL (2013) FoxP3 inhibits proliferation and induces apoptosis of gastric cancer cells by activating the apoptotic signaling pathway. ***Biochem Biophys Res Commun*** 430, 804-809.

Mears AJ, Jordan T, Mirzayans F, Dubois S, Kume T, Parlee M, fdfdfd and fdfd (1998) Mutations of the forkhead/winged-helix gene, FKHL7, in patients with Axenfeld-Rieger anomaly. ***Am J Hum Gene*** 63, 1316-1328.

Muggerud AA, Ronneberg JA, Warnberg F, Botling J, Busato F, Jovanovic J, Solvang H, Bukholm I, Borresen-Dale AL, Kristensen VN, Sørlie T and Tost J (2010) Frequent aberrant DNA methylation of ABCB1, FOXC1, PPP2R2B and PTEN in ductal carcinoma in situ and early invasive breast cancer. ***Breast Cancer Res*** 12, R3.

Myatt SS and Lam EW (2007) The emerging roles of forkhead box (Fox) proteins in cancer. ***Nat Rev Cancer*** 7, 847-859.

Natalwala A, Spychal R and Tselepis Ch (2008) Epithelial-mesenchymal transition mediated tumourigenesis in the gastrointestinal tract. ***World J Gastroenterol*** 14, 3792-3797.

Nishimura DY, Swiderski RE, Alward WL, Searby CC, Patil SR, Bennet SR, Kanis AB, Gastier JM, Stone EM and Sheffield VC (1998) The forkhead transcription factor gene FKHL7 is responsible for glaucoma phenotypes which map to 6p25. ***Nat. Genet*** 19, 140-147.

Peek Jr RM and Blaser MJ (2002) Helicobacter pylori and gastrointestinal tract adenocarcinomas. ***In Nat Rev Cancer*** 2, 28-37.

Peraldo-Neia C, Migliardi G, Mello-Grand M, Montemurro F, Segir R, Pignochino Y, Cavalloni G, Torchio B, Mosso L, Chiorino G and Aglietta M (2011) Epidermal growth factor receptor (EGFR) mutation analysis, gene expression profiling and EGFR protein expression in primary prostate cancer. ***BMC Cancer*** 11:31. doi:10.1186/ 1471-2407-11-31.

Pierrou, S, Hellqvist, M, Samuelsson, L, Enerback, S and Carlsson, P (1994) Cloning and characterization of seven human forkhead proteins: binding site specificity and DNA bending. ***EMBO J*** 13, 5002-5012.

Polyak K and Weinberg RA (2009) Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. ***Nat Rev Cancer*** 9, 265-273.

Ray PS, Wang J, Qu Y, Sim MS, Shamonki J, Bagaria SP, Ye X, LB, Elashoff D, Hoon DS, Walter MA, Martens JW, Richardson AL, Giuliano AE and Cui X (2010) FOXC1 is a potential prognostic biomarker with functional significance in basal-like breast cancer. ***Cancer Res*** 70, 3870-3876.

Seo S, Fujita H, Nakano A, Kang M, Duarte A and Kume T (2006) The forkhead transcription factors, Foxc1 and Foxc2, are required for arterial specification and lymphatic sprouting during vascular development. ***Dev Biol*** 294, 458-470.

Seo S, Singh HP, Lacal PM, Sasman A, Fatima A, Liu T, Schultz KM, Losordoa DW, Lehmannb OJ and Kumea T (2012) Forkhead box transcription factor FoxC1 preserves corneal transparency by regulating vascular growth. ***Proc Natl Acad Sci U S A*** 109, 2015-2020.

Shah MA and Kelsen DP (2010) Gastric cancer: a primer on the epidemiology and biology of the disease and an overview of the medical management of advanced disease. ***J Natl Compr Canc Netw*** 8, 437-447.

Squazzo SL, O'Geen H, Komashko VM, Krig SR, Jin VX, Jang SW, Margueron R, Reinberg D, Green R and Farnham PJ (2006) Suz12 binds to silenced regions of the genome in a cell-type-specific manner. ***Genome Res*** 16, 890-900.

Thiery JP (2002) Epithelial-mesenchymal transitions in tumour progression. ***Nat Rev Cancer*** 2, 442-454.

Wang L, Gu F, Liu CY, Wang RJ, Li J and Xu JY (2013) High level of FOXC1 expression is associated with poor prognosis in pancreatic ductal adenocarcinoma. ***Tumour Biol*** 34(2), 853-858.

Wei LX, Zhou RS, Xu HF, Wang JY and Yuan MH (2013) High expression of FOXC1 is associated with poor clinical outcome in non-small cell lung cancer patients. ***Tumour Biol*** 34(2), 941-946.

Xia L, Huang W, Tian D, Zhu H, Qi X, Chen Z, Zhang Y, Hu H, Fan D, Nie Y and Wu K (2012) Overexpression of forkhead box C1 promotes tumor metastasis and indicates poor prognosis in hepatocellular carcinoma. ***Hepatology*** 57(2), 610-624.

Yeh TS, Wu CW, Hsu KW, Liao WJ, Yang MC, Li AF, Wang AM, Kuo ML and Chi CW (2009). The activated Notch1 signal pathway is associated with gastric cancer progression through cyclooxygenase-2. ***Cancer Res*** 69, 5039-5048.

Yuasa Y (2003) Control of gut differentiation and intestinal-type gastric carcinogenesis. ***Nat Rev Cancer*** 3, 592–600.

Zhao R, Liu XQ, Wu XP, Liu YF, Zhang ZY, Yang GY, Guo S, Niu J, Wang JY and Xu KS (2010) Vascular endothelial growth factor (VEGF) enhances gastric carcinoma invasiveness via integrin alpha(v)beta6. ***Cancer Lett*** 287, 150-156.

Zhou Y, Kato H, Asanoma K, Kondo H, Arima T, Kato K, Matsuda T and Wake N (2002) Identification of FOXC1 as a TGFbeta1responsive gene and its involvement in negative regulation of cell growth. ***Genomics*** 80, 465-472.