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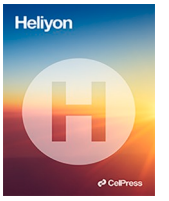

Research article

High expression of cytoplasmic FOXO3 protein associated with poor prognosis of rectal cancer patients: A study from Swedish clinical trial of preoperative radiotherapy to big database analysis

Weiyingqi Cui [a,1], Ning Xie [b,1], Eric W.-F. Lam [c], Victoria Hahn-Stromberg [d], Na Liu [a,b,***], Hong Zhang [e,**], Xiao-Feng Sun [a,*]

[a] Department of Oncology and Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden
[b] Department of Gastroenterology, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China
[c] Department of Surgery and Cancer, Imperial College London, Hammersmith Hospital, London, W12 0NN, United Kingdom
[d] Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden
[e] School of Medicine, Institute of Medical Sciences, Örebro University, Örebro, Sweden

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ABSTRACT

Introduction: Accumulating evidence has implicated a pivotal role for FOXO3, FOXM1 and SIRT6 in cancer progression. The majority of researches focused on the functions of these proteins in drug resistance, but their relationships with radiotherapy (RT) response remain unclear. In this study, we examined protein expression of FOXO3, FOXM1 and SIRT6 and their clinical significance in a Swedish rectal cancer trial of preoperative RT.

Methods: Expression of FOXO3, FOXM1 and SIRT6 protein was examined by immunohistochemistry in patient samples. Genetic analysis of FOXO3, FOXM1 and SIRT6 were performed by cBioportal and MEXPRESS database. Gene-gene network analysis was conducted using GeneMANIA. Functional enrichment analysis was performed based on LinkedOmics and Metascape online software.

Results: FOXO3 and FOXM1were mainly expressed in the cytoplasm in both normal and tumour tissues, and SIRT6 in both the cytoplasm and nucleus in normal and tumour tissues. FOXO3 and FOXM1 expression increased from normal mucosa to primary cancer ($P < 0.001$), while SIRT6 expression decreased from normal mucosa to primary cancer ($P < 0.001$). High FOXO3 expression correlated with late TNM stage ($P = 0.040$), distant metastasis ($P = 0.032$) and independently with disease free survival (DFS) in the RT patients (HR = 7.948; $P = 0.049$; 95% CI = 1.002–63.032) but not in non-RT patients ($P > 0.05$). Genetic analysis indicated that DNA methylation status contributed to FOXO3 overexpression. Functional enrichment analysis demonstrated that FOXO3 was closely related to metabolism-related signalling pathway which in turn associated with cancer radioresistance. Moreover, there were strong gene–gene interactions between FOXO3 and metabolism-related signalling.

Conclusions: Our findings suggest that FOXO3 may be a prognostic factor in rectal cancer patients with RT.

* Corresponding author. ;
** Corresponding author.
*** Corresponding author.Department of Oncology and Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden.
E-mail addresses: liunafmmu@163.com (N. Liu), hong.zhang@oru.se (H. Zhang), xiao-feng.sun@liu.se (X.-F. Sun).
[1] These authors contributed equally to the work.

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1. Introduction

Colorectal cancer is one of the most common cancers worldwide [1]. Although the treatments for colorectal cancer have been greatly improved in the last decades, one third of patients still display distant recurrence in 5 years according to the 10-year follow up of EOTRC trail [2]. Preoperative radiotherapy (RT) has been commonly used in the clinics as a standard routine treatment for advanced rectal cancer patients and has proven to have beneficial effects on lowering recurrence and increasing survival rates [3,4]. However, it has been also reported that RT can enhance cancer migration and invasion, which results in poorer prognosis in patients [5]. This indicates that the exact mechanisms underlying the therapeutic effects of RT as the standard therapy for rectal cancer patients have not been completely elucidated. In consequence, it is pertinent to provide better evidence to further support the positive effects of the RT for rectal cancer therapy, such as biomarkers to distinguish patients who would benefit from preoperative RT and improve survival.

Forkhead box (FOX) family proteins, function mainly as transcription factors, have been extensively investigated in a number of cellular processes [6]. Numerous studies have shown that two members of Forkhead family, FOXO3 and FOXM1, play extremely crucial roles in cancer progression [6]. FOXO3 (also previously known as FKHL1), belonging to the FOXO subfamily, has a controversial role in the progression of cancer, and is involved in regulating the cell cycle, inhibiting proliferation and programming cell death. Our previous study has shown that the de-activation of FOXO3 may depend on the phosphorylation by protein kinase B (also called Akt) [7], and the phosphorylated forms of FOXO3 are translocated from the nucleus to the cytoplasm. FOXM1 is proven to promote cell cycle progression, DNA damage repair and cell survival [8,9] upon DNA damage. In addition, FOXM1 can also work with FOXO3 in an axis [8]; both can bind to the same downstream target genes and antagonise the functions of each other. Meanwhile, as one of the key targets of FOXO3 and FOXM1, SIRT6, as a member from nicotinamide adenine dinucleotide (NAD)+-dependent sirtuin protein family, has also attracted lots of attention. Like FOXO3 and FOXM1, SIRT6 is involved in many crucial cellular processes including metabolism, genome stability maintenance and ageing, may also promote tumorigenesis and cancer progression [10].

Previous studies have focused on the involvement of FOXM1/FOXO3/SIRT6 network in caner development, chemoresistance and radioresistance among several cancer types, including breast, liver, and bladder cancer [7,10,11]. Hitherto, little is known about the expression patterns of FOXO3, FOXM1 and SIRT6 in rectal cancer patients with and without preoperative RT, therefore it is crucial to understand if the expression of these molecules is also involved in RT response in rectal cancer. Through investigating the patient materials from a Swedish rectal cancer clinical trial, in this study we are the first to identify FOXO3 as an independent prognostic factor in rectal cancer patients receiving RT. Our findings suggest that FOXO3 may be a reliable biomarker for optimising RT for rectal cancer patients in order to improve prognosis.

2. Materials and methods

2.1. Rectal cancer patients and materials

Patients were from the South-East Swedish Health Care region and participated in the randomized Swedish Rectal Cancer Trial of preoperative RT between 1987 and 1990 (Swedish Rectal Cancer Trial, 1997 [3]). Every participant signed the informed consent. The patient cohort of FOXO3 included 143 primary rectal adenocarcinomas, 124 normal mucosa specimens (112 corresponding to the primary cancer, *i.e.,* normal mucosa and primary cancer from the same patient) that were histologically free from cancer and taken from the margin of distant surgical resection, and 50 lymph node metastases (47 corresponding to the primary cancer, also in the irradiation field). Of the 143 patients (median age, 69 years), 79 underwent surgery alone and 64 received RT followed by tumour resection. RT was given with 25 Gy in 5 fractions within a median of 7 days (range, 4–12 days). Surgery was then carried out in a median of 3 days (range, 0–11 days) after RT. None of the patients received preoperative or adjuvant chemotherapy. The mean follow-up period was 105 months (range, 0–309 months), and information on local and distant recurrence; disease free survival and overall cancer-specific survival (OS) were obtained from patient medical records. The patient cohort of FOXM1 included 141 primary rectal adenocarcinomas, 103 normal mucosa specimens (85 corresponding to the primary cancer, *i.e.,* normal mucosa and primary cancer from the same patient) that were histologically free from cancer and taken from the margin of distant surgical resection, and 45 lymph node metastases (41 corresponding to the primary cancer, also in the irradiation field). Of the 141 patients (median age, 69 years), 77 underwent surgery alone and 64 received RT followed by tumour resection. All the rest of information is the same as FOXO3. The patient cohort of SIRT6 included 145 primary rectal adenocarcinomas, 120 normal mucosa specimens (84 corresponding to the primary cancer, *i.e.,* normal mucosa and primary cancer from the same patient) that were histologically free from cancer and taken from the margin of distant surgical resection, and 49 lymph node metastases (45 corresponding to the primary cancer, also in the irradiation field). Of the 145 patients (median age, 69 years), 80 underwent surgery alone and 75 received RT followed by tumour resection. All the rest information is the same as FOXO3. All the characteristics of the patients and tumours of these three factors are presented in Table 4 (FOXO3), Table S3 (FOXM1) and Table S4 (SIRT6).

2.2. Tissue samples and immunohistochemistry (IHC)

Expression of FOXO3, FOXM1 and SIRT6 protein was examined by IHC in 4 μm tissue microarray sections from paraffin-embedded surgical specimens. Sample sections were deparaffinized by immersing the slides twice in 100% xylene at room temperature for 10 min each. This was followed by incubating twice in 100% ethanol for 10 min each, and rehydrating with decreasing concentrations of ethanol (90% and 70%; vol/vol in water, 10 min each) before a final 5-min incubation in water. Antigen retrieval was carried out in a

target retrieval citrate buffer (pH 6.0) (Dako, Glostrup, Denmark) at 95 °C for 15 min. The sections were allowed to cool for 15 min and rinsed with phosphate-buffered saline (PBS) followed by incubation in 3% H_2O_2-methanol for 5 min to block the activity of endogenous peroxidase. After being washed in PBS, the sections were incubated with protein block (Dako) for 10 min to reduce nonspecific background staining. The sections were incubated with primary antibody overnight. After being washed in PBS, the sections were incubated with a secondary antibody, Envision System Labelled Polymer-HRP Anti-Rabbit (Dako) for 25 min. The sections were rinsed in PBS before reacting with Liquid DAB+ (Dako) to produce coloration. Finally, the sections were lightly counterstained with haematoxylin.

The immunostaining was scored by two independent observers based on the intensity and localization. Staining intensity in normal epithelial cells or tumour cells was graded according to the following criteria: 0 (no staining); 1 (weak staining = light yellow); 2 (moderate = yellow brown); 3 (strong = brown); and 4 (very strong = dark brown). The staining patterns were graded as cytoplasmic or nuclear. In case of discrepancy, a consensus score was reached after re-evaluation. For statistical analyses, cases scored less than 2 were considered as low-expressing group, and cases scored higher than 3 as high-expressing group (Fig. S1). The data regarding IHC expression of NF-κB, p53, p73, survivin, Cox2 and PPAR-delta was obtained at our laboratory on the same patient samples as in the present study. The used cut-off points were the same as previous corresponding publications [12–16].

2.3. cBioPortal database analysis

The cBio Cancer Genomics Portal (cBioPortal) (http://www.cbioportal.org/) provides visualization tools for more than 5000 tumour samples from 232 cancer studies in the TCGA database [17]. In this study, the Colorectal Adenocarcinoma (TCGA, Firehose Legacy, n = 640) cohort was analysed to explored the genetic alterations of FOXO3, FOXM1 and SIRT6.

2.4. MEXPRESS tool analysis

The MEXPRESS tool (https://mexpress.be) is a user-friendly online tool visualizing TCGA data, contains the information on mRNA expression, DNA methylation, clinical data as well as the relationships among these parameters [18]. The detail methylation locations of FOXO3 in colorectal cancer were assessed using the MEXPRESS.

2.5. GeneMANIA analysis

GeneMANIA (http://www.genemania.org) is a friendly web server for deriving hypotheses based on gene functions [19]. GeneMANIA was adopted to conduct a gene-gene interaction network for FOXO3, FOXM1 and SIRT6.

2.6. The Cancer Regulome tools and data analysis

The Cancer Regulome tools and data (http://explorer.cancerregulome.org/) from the TCGA dataset were performed to draw circus plots to show the genomic location of FOXO3 and its related-genes in colorectal cancer (n = 621). Spearman correlation was used to show the pairwise correlation between two genes. The circus plots only display the genes with *P*-values > log 10.

2.7. Functional enrichment analysis

The Spearman correlation analysis was conducted by the LinkedOmics database (http://www.linkedomics.org/) [20]. Spearman's correlation coefficient exceeding 0.4 indicates a good correlation between FOXO3 and its related genes. Metascape online software (http://metascape.org) was used to construct the interaction network of enrichment terms. All analyses were performed with default software parameters [21].

2.8. Statistical analyses

All statistical analyses were performed using STATISTICA software package (version 12.0; STATSOFT Inc., Tulsa, OK). McNemar's or Person χ^2 test was used to examine the significance of the differences in FOXO3/FOXM1/SIRT6 expression among normal mucosa, primary cancer and lymph node metastasis, as well as the association of FOXO3/FOXM1/SIRT6 expression with clinicopathological or biological variables. The survival curves were plotted using Kaplan–Meier analysis and the differences between the curves were calculated by Log rank test. Univariate and multivariate analyses were performed by Cox proportional hazards regression analysis (likelihood ratio test). All tests were two sided and *P*-values <0.05 were considered statistically significant.

3. Results

3.1. Expression of FOXO3, FOXM1 and SIRT6 proteins in normal mucosa, primary cancer, and lymph node metastasis from rectal cancer patients

As shown in Fig. 1A, FOXO3 was predominantly expressed in the cytoplasm of epithelial cells in the normal mucosa (left panel), primary cancer (middle panel) and lymph node metastasis (right panel). In the non-RT group, high levels of FOXO3 expression were

detected in primary cancer (90%) when compared with distant normal mucosa (14%) and adjacent normal mucosa (18%) respectively (both $P < 0.001$), and there were no statistically significant changes in FOXO3 expression levels from primary cancer (90%) to metastasis (82%) (Fig. 1B). In the RT group, the proportion of cells with high FOXO3 expression was significantly increased in primary

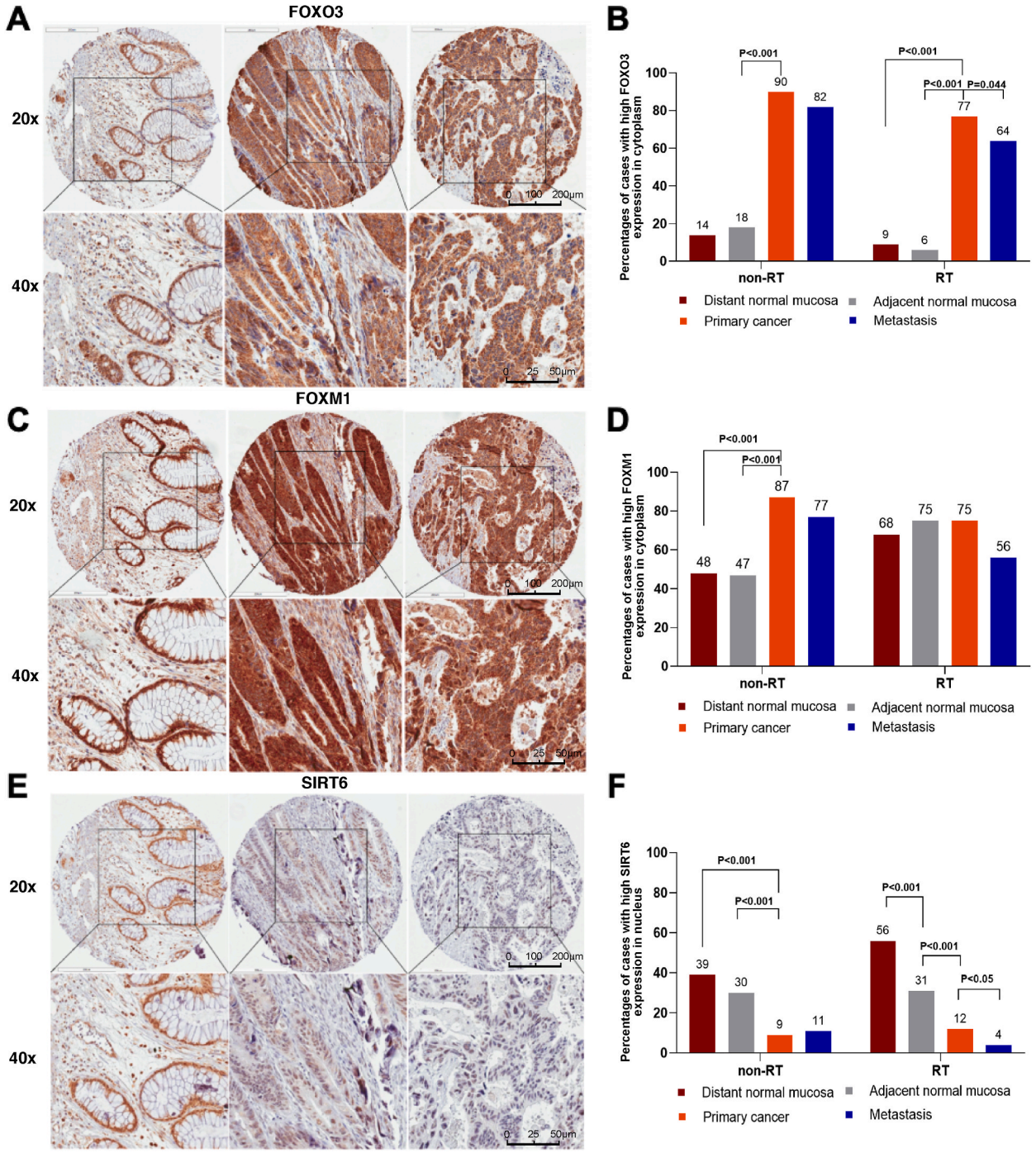


Fig. 1. Expression of FOXO3/FOXM1/SIRT6 protein in various tissues from the rectal cancer patients. Immunohistochemical staining for the expression of FOXO3 (A), FOXM1 (C) and SIRT6 (E) in the normal mucosa (left panel), primary tumour (middle panel) and lymph node metastasis (right panel) in both low and high power, respectively. In the top rows, the scale bars indicate 200 μm, whereas in the bottom rows, the scale bars correspond to 50 μm. Magnification 20x and 40x. Percentages of the cases with high expression levels of FOXO3 (B), FOXM1 (D) and SIRT6 (F) in the distant normal mucosa, adjacent normal mucosa, primary cancer and lymph node metastasis in non-radiotherapy (non-RT) and radiotherapy (RT) rectal cancer patients.

cancer (77%) compared with distant normal mucosa (9%) and adjacent normal mucosa (6%), respectively (both $P < 0.001$), while it was reduced from primary cancer (77%) to metastasis (64%) ($P = 0.044$; Fig. 1B).

FOXM1 was found mainly in the cytoplasm of the epithelial cells in the normal mucosa, primary cancer, and lymph node metastasis (Fig. 1C). In the non-RT group, the proportion of cells with high FOXM1 expression was significantly augmented in primary cancer (87%) compared with distant normal mucosa (48%) and adjacent normal mucosa (47%) respectively (both $P < 0.001$), but there were no statistically significant changes going from primary cancer (87%) to metastasis (77%) (Fig. 1D). However, there were no significant changes in FOXM1 expression h among distant normal mucosa (68%), adjacent normal mucosa (75%), primary cancer (75%) and metastasis (58%) in the RT patients (all $P > 0.05$).

SIRT6 was found to express in both the cytoplasm and nucleus of the epithelial cells in the normal mucosa, as well as cancer cells in primary cancer and lymph node metastasis (Fig. 1E). Since SIRT6 is considered to function predominantly in the nucleus, the nucleic staining was evaluated further. In the non-RT group, the frequency of nuclear SIRT6 expression was decreased significantly from the distant normal tissue (39%) to adjacent normal tissue (30%) and to primary cancer (9%) (both $P < 0.001$), and there were no significant changes in nuclear SIRT6 expression between primary cancer (9%) and metastasis (11%). In the RT group, the frequency of high SIRT6 expression was decreased significantly from distant normal mucosa (56%), to adjacent normal mucosa (31%) and primary cancer (12%) (both $P < 0.001$), and to metastasis (5%) (all $P < 0.05$) (Fig. 1F).

3.2. Relationship of FOXO3, FOXM1 and SIRT6 protein expression with clinicopathological variables in rectal cancer patients

We next investigated the significance of FOXO3 and FOXM1 expression in the primary cancer in relation to various clinicopathological factors (Table 1). FOXO3 expression was found to be significantly associated with the late TNM stages ($P = 0.040$), recurrence (local + distant recurrence, $P = 0.018$) and distant recurrence ($P = 0.032$) in RT patients, and only recurrence ($P = 0.028$) in non-RT patients. There was no relationship between FOXO3 and gender, age, histological type, lymphovascular invasion or local recurrence (all $P > 0.05$, Table 1) in either RT or non-RT patients. The cytoplasmic FOXM1 expression was not related to any of the above-mentioned clinicopathological variables in either RT or non-RT patients ($P > 0.05$). SIRT6 expression was also not correlated with any of the above-mentioned clinicopathological variables in either RT or non-RT patients ($P > 0.05$).

We further analysed the relationship between FOXO3 expression and survival in non-RT and RT patients, respectively. In the non-RT group, there was no statistically significance between the high and low FOXO3 expression regarding cancer-specific survival (CSS) ($P = 0.119$; Fig. 2A). However, in the RT group, patients with high FOXO3 expression were found to have poor CSS compared with patients with low FOXO3 expression ($P = 0.047$, Fig. 2B). Moreover, those patients with high FOXO3 expression had poor disease-free survival (DFS) in both the non-RT group ($P = 0.049$, Fig. 2C) and RT group ($P = 0.022$, Fig. 2D). The significance between high and low FOXO3 expression in DFS still existed, after adjusting for gender, age, TNM stage, histological type, surgical type, and resection margin

Table 1
FOXO3 expression in primary cancers in relation to clinicopathological variables in rectal cancer patients.

Variables	Non-RT			RT		
	FOXO3 expression			FOXO3 expression		
	Low (%)	High (%)	p-value	Low (%)	High (%)	p-value
Gender			0.265			0.816
Male	3 (7)	39 (93)		8 (22)	29 (78)	
Female	5 (15)	28 (85)		4 (19)	17 (81)	
Age (years)			0.145			0.244
≤69	6 (16)	32 (84)		9 (26)	26 (74)	
>69	2 (5)	35 (95)		3 (13)	20 (87)	
Tumour stage			0.096			**0.040**
I	3 (14)	18 (86)		7 (39)	11 (61)	
II	4 (21)	15 (79)		4 (19)	17 (81)	
III	1 (3)	34 (97)		1 (5)	18 (95)	
Histological type			0.289			0.662
Non-mucinous	32 (50)	32 (50)		32 (63)	19 (27)	
Mucinous	8 (67)	4 (33)		7 (70)	3 (30)	
Lymphovascular invasion			0.474			0.650
Periphery	11 (61)	7 (39)		8 (50)	8 (50)	
Inner tumour area	22 (59)	15 (41)		17 (65)	9 (35)	
Invasive margin	6 (46)	7 (54)		10 (71)	4 (29)	
Negative cases	4 (36)	7 (64)		7 (64)	4 (36)	
Recurrence			**0.028**			**0.018**
No	7 (18)	31 (82)		11 (31)	25 (69)	
Yes	1 (3)	36 (97)		1 (5)	21 (95)	
Local recurrence			0.093			0.290
No	8 (14)	49 (86)		12 (22)	42 (78)	
Yes	0	18 (100)		0	4 (100)	
Distant recurrence			0.124			**0.032**
No	7 (15)	40 (85)		11 (29)	27 (71)	
Yes	1 (4)	27 (96)		1 (5)	19 (95)	

in the RT group (Table 2). The patients with high FOXO3 expression were 7.948 times more likely to have disease recurrence than patients with low FOXO3 expression (HR, 7.948; P = 0.049; 95% CI, 1.002–63.032). In addition, we found that the female patients were less likely to relapse compared to male patients in multivariate analysis (HR, 0.190; P = 0.014; 95% CI, 0.051–0.711). There was no significance in the non-RT group regarding either univariant or multivariant factors mentioned above; however, significances were found in multivariant analysis in CSS (HR, 8.717; P = 0.006; 95% CI, 1.842–41.261) and DFS (HR, 4.854; P = 0.030; 95% CI, 1.162–20.275) regarding histological types. For the study above, we accounted only for tumours stages I-III, since stage IV may affect the prognosis beyond RT and biomarkers.

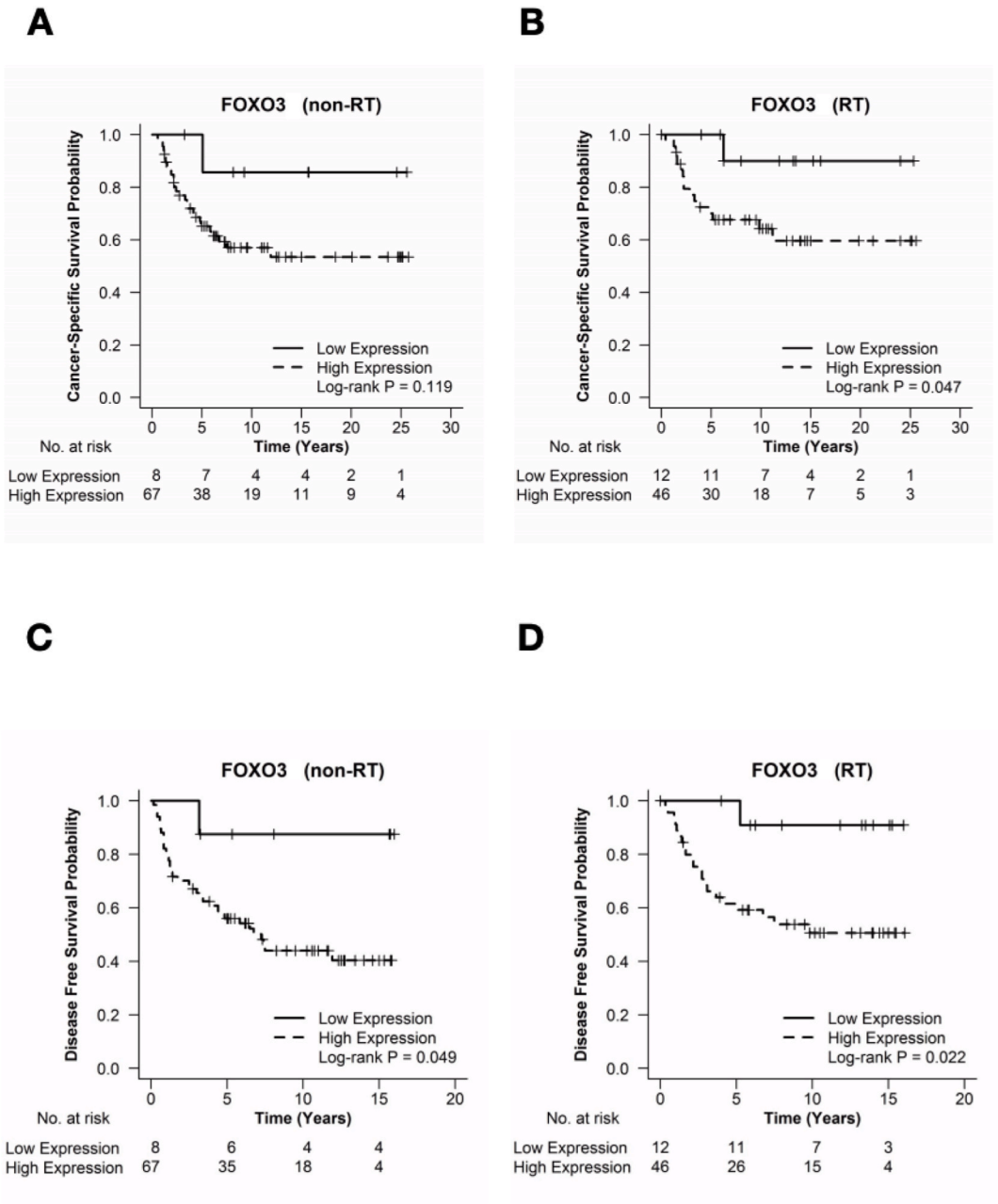


Fig. 2. The relationship between FOXO3 expression and survival in radiotherapy (RT) and non-radiotherapy (non-RT) rectal cancer patients. Cancer-specific survival analysis showed that the cancer-specific survival probability was significantly different in the rectal cancer patients between high and low FOXO3 expression in non-RT (A) and RT (B) groups. Disease free survival analysis revealed that rectal cancer patients with high and low FOXO3 expression was statistically different in non-RT (2C) and RT (2D) rectal cancer patients.

3.3. Relationship of FOXO3 protein expression with biological factors in rectal cancer patients

As shown in Table 3, the relationships of FOXO3 expression with biological factors have previously been examined on the same patient cohort at our laboratory. In the RT group, FOXO3 expression in the primary tumours was positively correlated with FOXM1 ($P = 0.003$) and cytoplasmic phospho-NF-κB at Serine 536 ($P = 0.049$). There was no statistical significance between FOXO3, and SIRT6, p53, p73, survivin, Cox-2 or PPAR-delta (all $P > 0.05$). In the non-RT patients, FOXO3 was also positively correlated with NF-κB expression ($P = 0.04$). However, there were no significant correlations between FOXO3 and the other biological factors mentioned above ($P > 0.05$).

3.4. Gene-gene interaction networks among FOXO3, FOXM1 and SIRT6 in colorectal cancer

Furthermore, we constructed the gene-gene network and function analysis using the GeneMANIA database. As showed in Fig. 3, FOXO3, FOXM1, SIRT6 and genes in NF-κB pathways have close relationships in the gene-gene interaction networks, such as co-expression, shared protein domains, co-localization, pathway, and genetic interaction. Evidently, they have genetic and physical interactions, and are involved similar pathways with other genes. Moreover, they also share similar protein kinases and diacylation as well as DNA repair and cell cycle G2/M transition.

3.5. The genetic alteration of FOXO3, FOXM1 and SIRT6 in colorectal cancer

In order to understand comprehensively the expression profiles of FOXO3, FOXM1 and SIRT6 in colorectal cancer, we analysed their genetic alterations by cBioPortal. The mutation ratios of FOXO3, FOXM1 and SIRT6 were found to be 7%, 6% and 2.9%, respectively (Fig. 4A). And their alteration frequencies in different subtypes of colorectal cancer were also examined (Fig. 4B). The analysis revealed that higher frequencies of FOXO3, FOXM1 and SIRT6 mRNA overexpression were commonly found in various subtypes of colorectal cancer. The FOXM1 mRNA overexpression was associated with relatively higher copy number amplification. The details concerning mutations of FOXO3 and FOXM1 in colorectal cancer were also analysed and shown in Fig. 4C. However, the analysis also demonstrated that the upregulation of FOXO3 and SIRT6 expression was not resulted from gene amplification.

3.6. DNA methylation status of FOXO3 in colorectal cancer

Dysregulation of the FOXO3 can be due to its DNA methylation status. As a result, further evaluation for DNA methylation status of FOXO3 gene in colorectal cancer was carried out and shown in Table S1. There was a negative correlation between mRNA expression and DNA methylation of FOXO3 ($R \geq 0.5$, $P < 0.05$). Specific methylation site analyses of FOXO3 were performed additionally based on MEXPRESS dataset. This indicated that distinct DNA methylation sites might regulate the FOXO3 expression.

3.7. Functional analysis of FOXO3-related genes in colorectal cancer

To uncover the mechanisms underlying the significant prognostic value of FOXO3 in colorectal cancer, we further explored the possible molecular functions of FOXO3 based on TCGA datasets. The circus plot in Fig. 5A and the volcano plot in Fig. 5B showed the genomic location of FOXO3 and all FOXO3-associated genes in colorectal cancer. As shown in Fig. 5C and D, the heat maps showed the genes positively and negatively associated with FOXO3 in colorectal cancer, respectively.

Table 2
Univariate and multivariate analysis of FOXO3, gender, age, stage, differentiation, surgical type and resection margin in relation to survival of rectal cancer patients in RT group.

Variables[a]	CSS				DFS			
	Univariant analysis		Multivariant analysis		Univariant analysis		Multivariant analysis	
	HR (95%CI)	*p*-value	HR (95%CI)	*p*-value	HR (95%CI)	*p*-value	HR (95%CI)	*p*-value
FOXO3 expression (high vs. low)	5.434 (0.720–41.026)	**0.047**	4.236 (0.527–34.016)	0.174	7.344 (0.987–54.638)	**0.022**	7.948 (1.002–63.032)	**0.049**
Gender (female vs. male)	0.507 (0.201–1.279)	0.154	0.396 (0.101–1.553)	0.184	0.556 (0.247–1.249)	0.154	0.190 (0.051–0.711)	**0.014**
Age (>69 vs.≤69)	0.801 (0.350–1.833)	0.598	0.640 (0.213–1.924)	0.427	0.714 (0.340–1.501)	0.374	0.594 (0.230–1.534)	0.282
TNM stage (III vs. I + II)	6.130 (2.609–14.397)	<0.001	6.500 (2.121–19.918)	**0.001**	6.945 (3.219–14.983)	**<0.001**	8.843 (3.140–24.901)	**<0.001**
Histological type (mucinous vs. non-mucinous)	1.468 (0.345–6.249)	0.603	0.000 (0.000–19.918)	1.000	1.101 (0.262–4.624)	0.895	0.000 (0.000–24.901)	1.000
Resection margin (positive vs. negative)	1.391 (0.327–5.926	0.65	0.435 (0.040–4.762)	0.496	1.064 (0.253–4.470	0.933	0.181 (0.017–1.935	0.157

Table 3
FOXO3 expression in the primary rectal tumour in relation to biological variables.

Variables	Non-RT			RT		
	FOXO3 expression			FOXO3 expression		
	Low (%)	High (%)	p-value	Low (%)	High (%)	P-value
NFκBp65			0.653			**0.049**
Low	0	2 (100)		3 (50)	3 (50)	
High	7 (3)	69 (97)		9 (6)	46 (94)	
FOXM1			0.317			**0.003**
Low	0	10 (100)		7 (50)	7 (50)	
High	6 (14)	59 (86)		6 (13)	40 (87)	
p53			0.951			0.097
Low	7 (88)	60 (88)		11 (20)	44 (80)	
High	1 (12)	8 (12)		3 (50)	3 (50)	
p73			0.163			0.068
Negative	8 (14)	48 (86)		6 (35)	11 (65)	
Positive	0	12 (100)		4 (29)	27 (71)	
Survivin			0.847			0.505
Low	5 (12)	36 (88)		6 (22)	21 (78)	
High	1 (10)	9 (90)		3 (33)	6 (67)	
Cox2			0.463			0.175
Low	2 (8)	22 (92)		3 (14)	19 (86)	
High	5 (15)	29 (85)		7 (30)	16 (70)	
PPAR-delta			0.767			0.069
Low	6 (10)	56 (90)		14 (28)	36 (72)	
High	1 (7)	13 (93)		0	9 (100)	
SIRT6			0.384			0.861
Negative	7 (10)	64 (90)		12 (22)	42 (78)	
Positive	0	7 (100)		2 (25)	6 (75)	

Table 4
Characteristics of patients and tumours.

Characteristics	Non-RT (%)	RT (%)	P-value
Gender			0.502
Male	45 (57)	40 (63)	
Female	34 (43)	24 (37)	
Age (years)			0.371
≤69	41 (52)	38 (59)	
>69	38 (48)	26 (41)	
Stage			0.268
I	21 (27)	18 (28)	
II	19 (24)	21 (33)	
III	35 (44)	19 (30)	
IV	4 (5)	6 (9)	
Histological type			0.923
Non-Mucinous	64 (84)	51 (84)	
Mucinous	12 (16)	10 (16)	
Number			0.518
Single	71 (86)	52 (84)	
Multiple[a]	10 (12)	10 (16)	
Unknown	2 (2)	0 (0)	
Resection margin			0.918
Negative	75 (95)	61 (95)	
Positive	4 (5)	3 (5)	
To anal verge (cm)			
Mean	7.341	8.656	

[a] Previous colorectal cancer and/or other types of tumours before the present rectal cancer.

GO (Gene Ontology) enrichment analysis indicated that the FOXO3-associated genes were significantly linked to several metabolism-related biological processes (Fig. 6A and Supplementary Table 3), such as generation of precursor metabolites and energy (gene ratio = 23/522, Log (p-value) = −16.627), ATP metabolic process (gene ratio = 18/311, Log (p-value) = −15.068) and ribonucleotide metabolic process (gene ratio = 18/311, Log (p-value) = −15.068). To further capture the internal associations among the terms, the top 20 enriched clusters were rendered as a network plot using Metascape online tools. A Kappa similarity >0.3 was considered as a good connection (Fig. 6B and C). Consistently, as shown in Fig. 6D and Table S2, Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis also demonstrated that FOXO3 was highly associated with metabolism-related signal pathways: Carbon metabolism (gene ratio = 5/114, Log (p-value) = −3.940215054) and Metabolism of xenobiotics by cytochrome P450 (gene

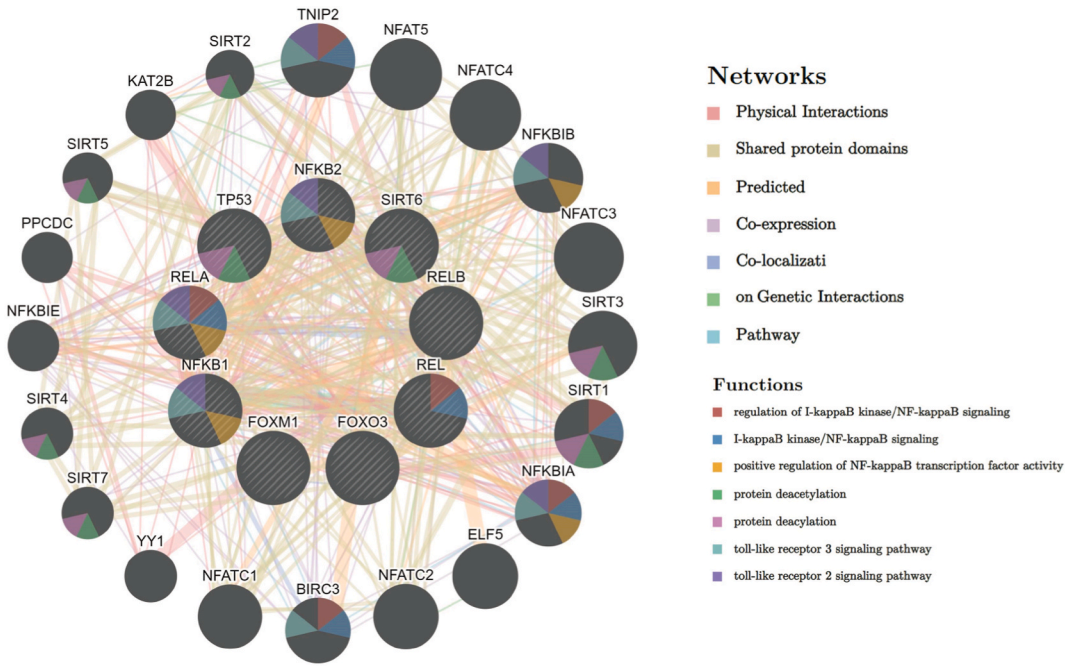


Fig. 3. Gene-gene interaction networks among FOXO3, FOXM1, SIRT6 and genes in NF-κB signalling in colorectal cancer. Each node represents an individual gene. The inter-node connection lines represent the types of gene-gene interactions and colour of the lines represents the types of interactions. The colour nodes represent the possible functions of respective genes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

ratio = 3/76, Log (p-value) = −2.407652205). Moreover, a gene–gene interaction network performed by GeneMANIA database also showed that there are several interactions between FOXO3 and genes involved in cell metabolism, including co-expression, co-localization, and genetic interaction (Fig. 6E).

4. Discussion

Our study is the first to concentrate on the relationship between high cytoplasmic expression FOXO3 and tumour progression in rectal cancer patients with preoperative RT. FOXO3 protein expression was significantly higher in primary tumours compared to normal tissues regardless of RT or non-RT status. Furthermore, higher FOXO3 expression in primary tumour was significantly associated with late TNM stages and distant recurrence in RT patients. Even more importantly, the patients whose tumours expressed high FOXO3 with RT had a worse prognosis compared to patients with tumours having low FOXO3 expression, independently of gender, age, TNM stage, histological type, surgical type, and resection margin. A similar result has previously been shown in bladder cancer patients [11]. Our findings suggest that FOXO3 can be a potential prognostic biomarker for preoperative RT-associated survival in rectal cancer patients.

Our further genetic analyses indicate that the DNA methylation status is involved in misregulation of FOXO3 expression in colorectal cancer. It is generally accepted that DNA methylation is a major epigenetic process that plays a critical role in different stages of evolution and disease development, including cancer progression. A prior study reported that FOXO3 expression was upregulated in breast cancer due to DNA hypomethylation [22]. In agreement, a genome DNA methylation analysis of active pulmonary tuberculosis also found that the patients with MRPS18B/FOXO3 hypomethylation showed lower than one-year survival [23]. Overall, our findings suggest that DNA hypomethylation is involved in the FOXO3 upregulation and the associated poor prognosis in colorectal cancer patients.

To explore further the FOXO3 function networks, we analysed the relationships of FOXO3 expression with other biological factors examined previously in the same patient cohort from our laboratory. Interestingly, our results indicated that FOXO3 expression in primary tumours was positively correlated with FOXM1 and the cytoplasmic phospho-NF-κB in the RT-group. Because of pleiotropic oncogenic role of NF-κB in a variety of cancer types, it is logically to speculate that further unappreciated functions of FOXO3 in cancer progression exist and the detailed mechanisms require further investigation.

The functions of FOXO3 in cancer progression, RT response and prognosis are controversial. In general, the FOXO3 has been considered as a suppressor in primary tumour growth through promoting apoptosis and/or restricting cell cycle progression. However, phosphorylation by kinases, particularly PI3-K/PKB signalling pathway, ERK, IKB kinase and serum and glucocorticoid-regulated kinase can promote FOXO3 nuclear to cytoplasmic shuttling, which may subsequently alter its normal biological functions in cancer progression. In chronic myeloid leukaemia, although FOXO3 activation can provoke Bim-induced apoptosis in Bcr-Abl-expressing

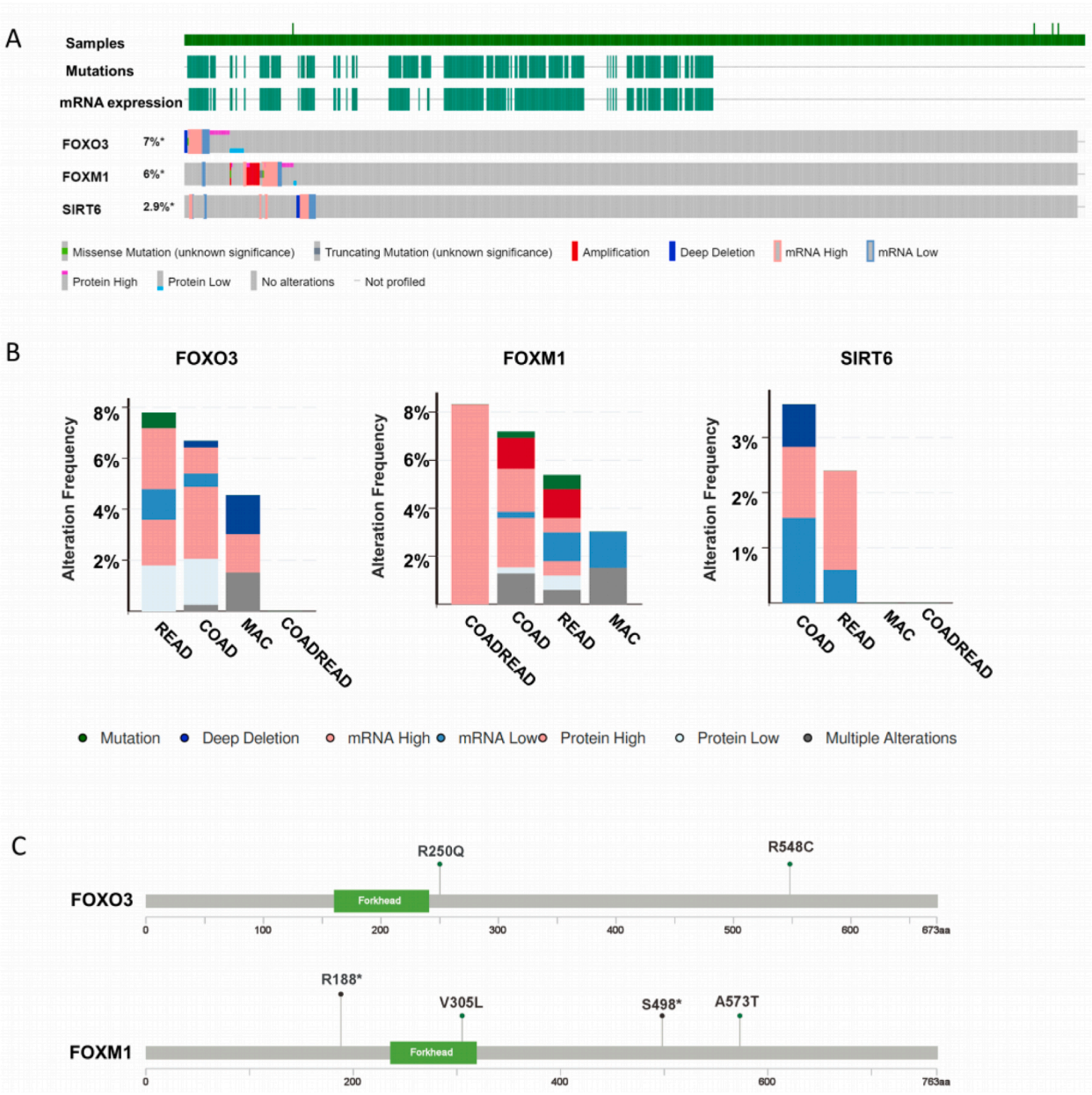


Fig. 4. Genetic alterations of FOXO3, FOXM1 and SIRT6 in colorectal cancer. The OncoPrint analysis summaries genetic variations of FOXO3, FOXM1 and SIRT6 in TCGA dataset (A). Alteration frequencies of *FOXO3* in various subtypes of the cancers. COAD: Colon Adenocarcinoma; READ: Rectal Adenocarcinoma; MAC: Mucinous Adenocarcinoma of the Colon and Rectum; COADREAD: Colorectal Adenocarcinoma (B). Detail mutation information of FOXO3 and FOXM1 in colorectal cancer (C).

cells through treatment with the Bcr-Abl inhibitor STI571 [24], continuous FOXO3 activation induced by doxorubicin have also been found to lead to drug resistance and cell survival via stimulating PIK3CA and ABCB1 expression [25]. Conversely, there is also evidence in colorectal cancer that knockdown of FOXO3 reduced MEK/MAPK phosphorylation and anchorage-independent growth, significantly decreasing the sensitivity to cetuximab in cells harbouring mutant, but not wild-type, KRAS [26]. As a candidate therapeutic strategy in pancreatic ductal adenocarcinoma, FOXO3 inhibition has been shown to suppress CD44 expression and cancer stem cell properties via the signalling axis of FOXO3/LKB1/AMPK/PGC-1β/PDHA1/CD44 [27,28]. Collectively, these functions of FOXO3 in cellular detoxification [29], the development of drug-resistance [25,30] and the feedback-regulation on PI3K/PKB-activity [31] also support a tumour promoting role for FOXO3. Nevertheless, irrespective of whether FOXO3 functions as an oncogene or a tumour suppressor, it is reasonable to propose that FOXO3 is crucial molecule in various aspects of cancer development, especially in drug sensitivity and resistance. In consequence, it is important to understand its role and expression in cancer treatment in order to uncover

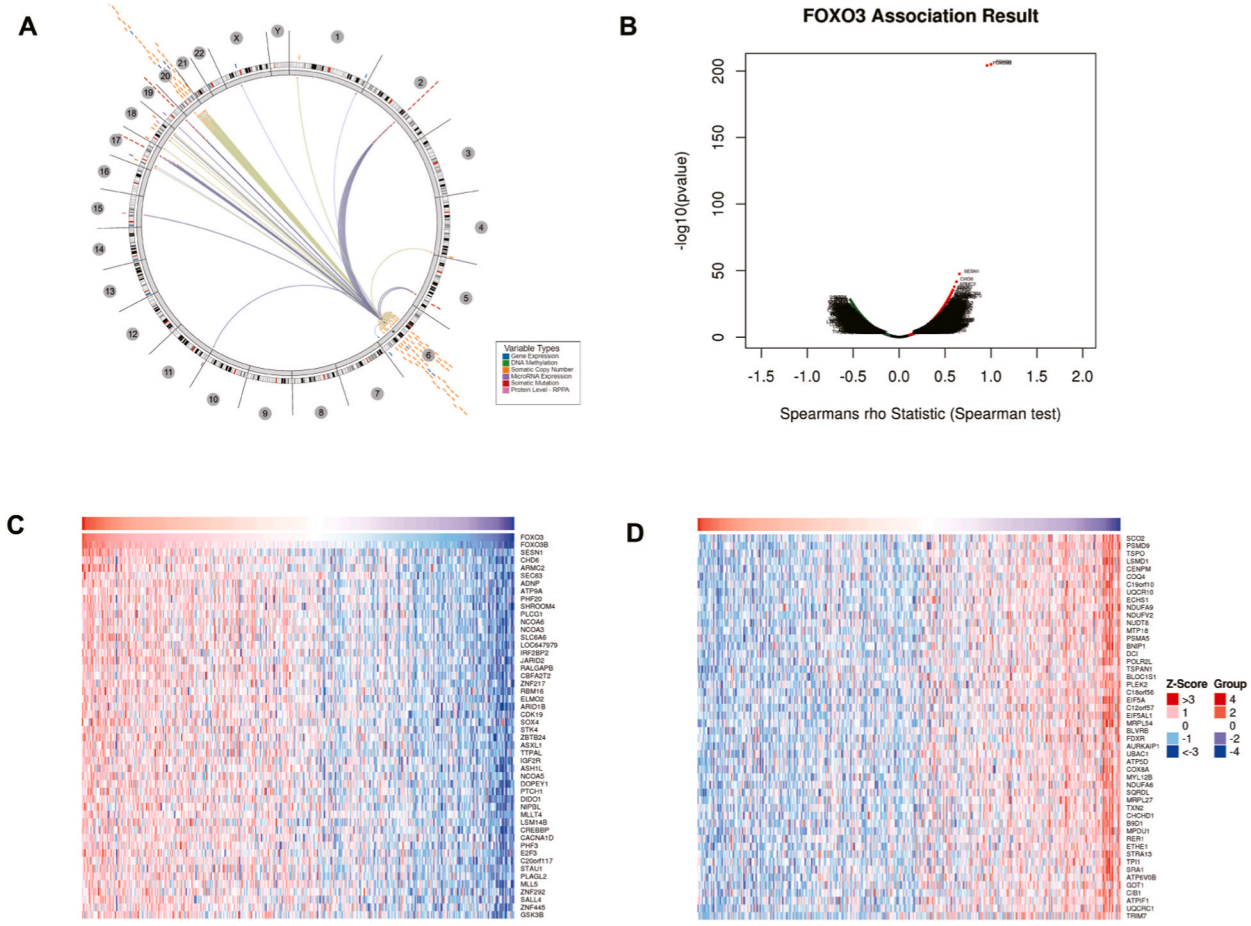


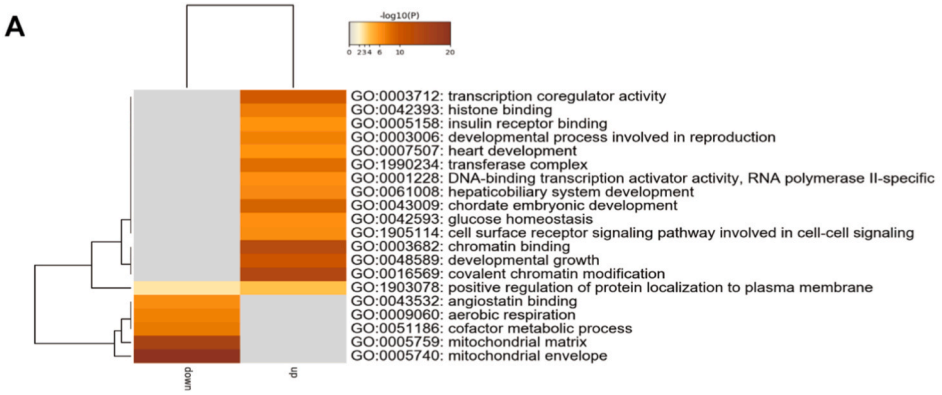
Fig. 5. Correlation analysis of FOXO3-related genes in colorectal cancer. Top 20 GO enrichment terms are listed in the bar graph (A). A volcano plot of FOXO3-related genes (B). Heat map showing the genes positively associated with FOXO3 (C). heat map revealing the genes negatively associated with FOXO3 (D).

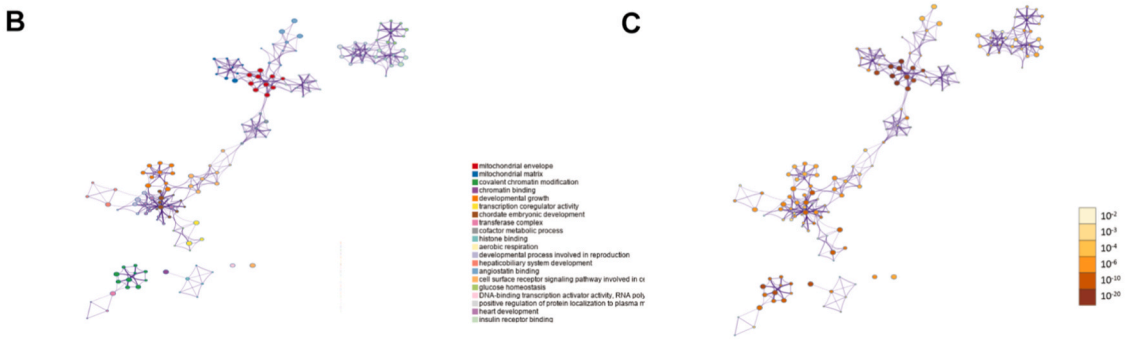
treatment strategies for overcoming chemotherapeutic drug resistance.

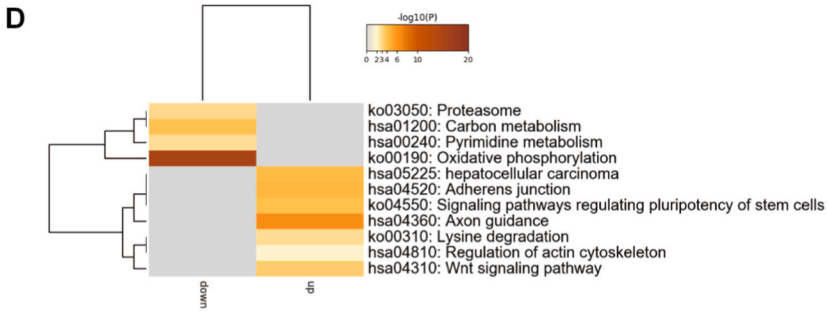
As mentioned previously, FOXM1 has been proven to be involved in a number of crucial processes in oncogenesis and cancer progression [6], such as stem cell expansion, epithelial-mesenchymal transition, and drug resistance [11]. Patients with high levels ofFOXM1expression have significantly poorer survival compared to patients with low FOXM1 expression, which is in concordance with previous studies in lung cancer [31] and glioblastoma [32] radioresistance pathway is a central actor of GBM treatment resistance and a key target to inhibit in the aim to increase the sensitivity of GBM to the RT. Multivariate analysis indicated that FOXM1 is an independent prognostic factor for disease-free survival in male breast cancer [6,32]. At odds with these results, we found that there was no significant correlation between FOXM1 and survival in our patient samples, which may due to the differences in patient samples/resources available or/and cancer types.

SIRT6 has also been observed to be a downstream target of FOXO3. SIRT6 has been reported to be able to restrict cancer survival and inhibit tumour prognosis via impairing in collaboration with HIF1α and p53 [33] and repair DNA damage [10]. In our patient samples, nuclear SIRT6 expression decreased from normal tissues to tumours and metastases in the lymph nodes. Even in the patients with non-small cell lung cancer, low nuclear and high cytoplasmic SIRT6 expression has been found to be associated with poor survival [33]. However in our study there was no significant correlation between SIRT6 and survival in rectal cancer, indicating that SIRT6 may play different roles in distinct cancer types, and its expression might not directly affect rectal cancer progression in relation to RT.

In the present study, we found that FOXO3 and FOXM1 expression in the cytoplasm of both the normal mucosa and cancer tissue. However, SIRT6 protein was expressed in the both cytoplasm and nucleus of the normal mucosa and cancer tissue. The expression of FOXO3, FOXM1 and SIRT6 protein was significantly different between normal mucosa, primary tumour and lymph node metastasis in RT and/or non-RT patients. Moreover, FOXO3 expression was statistically related to TNM stages, recurrence and prognosis, as well as NF-κB p65 in RT patients [33]. Further enrichment analysis based on TCGA dataset also demonstrated that FOXO3 was involved in several metabolism-related signalling in colorectal cancer, which was reported to play a crucial role in radioresistance [34]. These findings strongly suggest that FOXO3, FOXM1 and SIRT6 play various roles in rectal cancer, and FOXO3 was the core molecule involved in the rectal cancer progression, RT response and prognosis (Fig. 7).







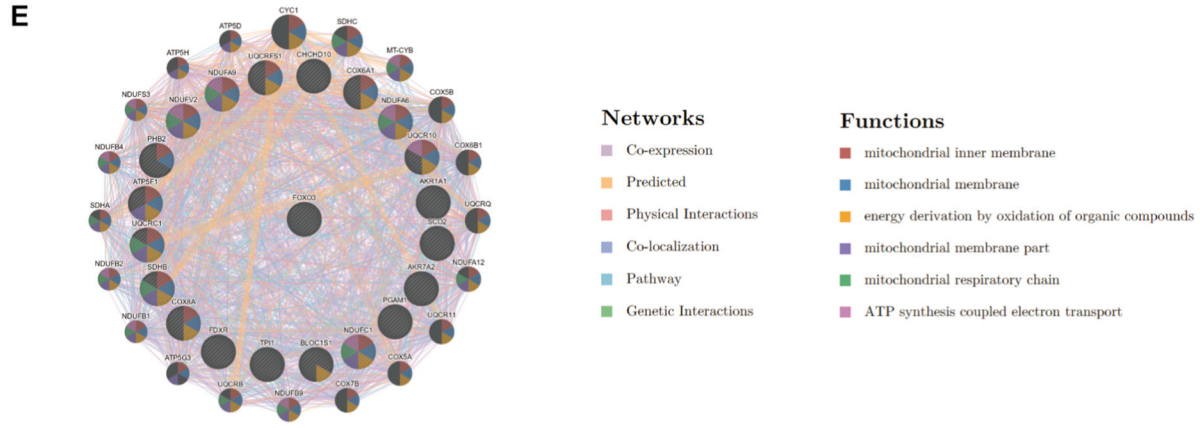


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Fig. 6. Functional enrichment analysis of FOXO3-associated genes in colorectal cancer. We select the term between upregulated and downregulated groups with the best p-value within each cluster as its representative term and display them in a dendrogram. The heatmap cells are coloured by their p-values, white cells indicate the lack of enrichment for that term in the corresponding gene list. Top 20 GO enrichment terms are listed in the dendrogram (A). Network of the top 20 GO enriched terms coloured by clusters (B). Network of the top GO 20 enriched terms coloured by P-value (C). Top 20 KEGG enrichment terms are listed in the dendrogram (D). Gene-gene interaction networks between FOXO3 and the genes in metabolism-related pathways in colorectal cancer. Each node represents an individual gene. The inter-node connection lines represent the types of gene-gene interactions and colours of the lines represent the types of interactions. The colour nodes represent the possible functions of respective genes (E). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

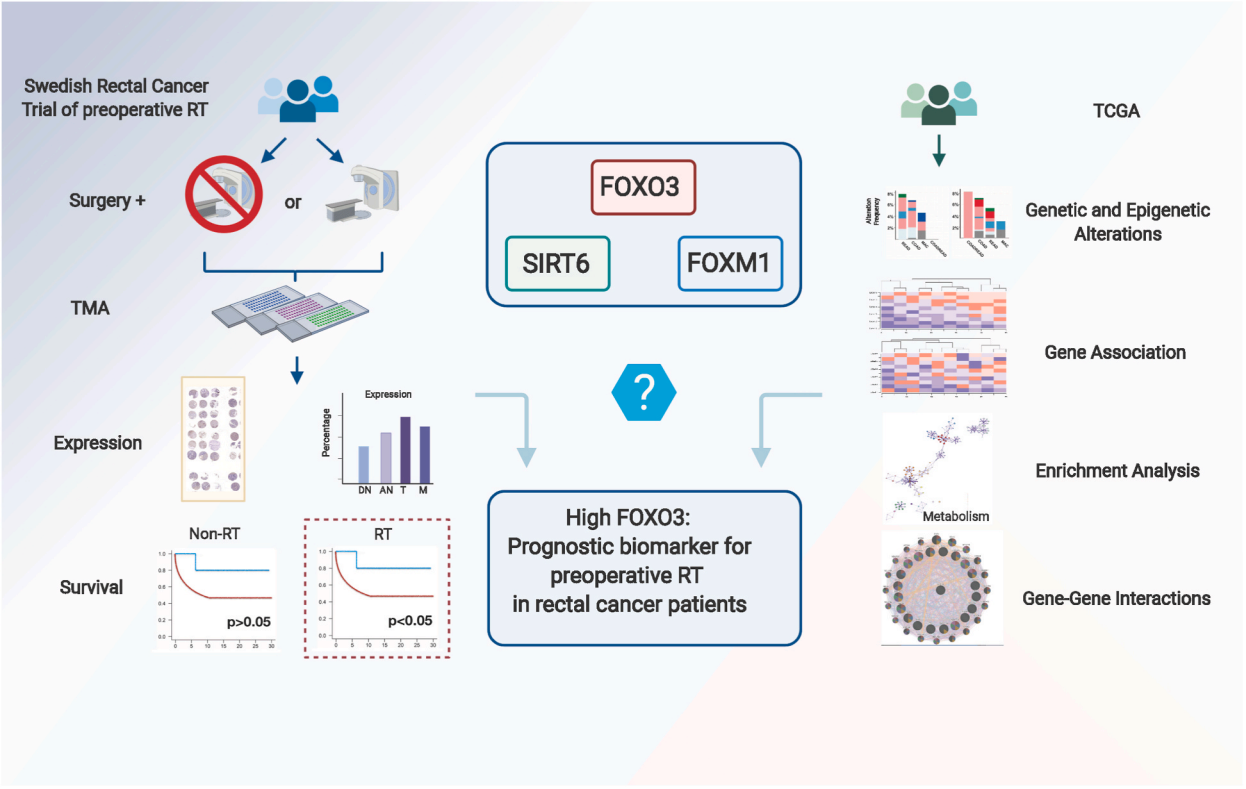

Fig. 7. Schematic diagram of research flow.

There are some limitations in the present study. We have relatively small numbers of the cases. Besides, due to the understandings of pre-operative radiotherapy at the time, the surgery methods and therapeutic tactics are different from the ones to dates. However, our study indicates the importance of FOXO3as an independent prognostic factor in rectal cancer patients with preoperative radiotherapy, and this evidence may reveal valuable information for developing more effective strategies of therapy in the future, which will improve the therapy response and, ultimately, clinical outcomes and prognosis.

5. Conclusions

In the present study, high FOXO3 expression was strongly associated with late TNM stages, distant recurrence, and poor prognosis, independently of gender, age, TNM stage, histological type, surgical type and resection margin in RT patients, but not in non-RT patients. In conclusion, FOXO3 is the key biomarker for predicting RT response and prognosis in rectal cancer patients.

Author contribution statement

Weiyingqi Cui; Ning Xie: Performed the experiments; Analysed and interpreted the data; Wrote the paper.
Eric. W.-F. Lam: Analysed and interpreted the data.
Victoria Hahn-Stromberg: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.
Na Liu: Conceived and designed the experiments; Performed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Hong Zhang; Xiao-Feng Sun: Conceived and designed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

The authors do not have permission to share data.

Ethics approval and consent to participate

This study was approved by the institutional ethics committee at Linkoping University. The informed consent was signed by each participant.

Consent for publication

The consent forms were signed by every participant and will be provided upon request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e15342.

Abbreviations

RT	radiotherapy
RFS	disease free survival
FOX	Forkhead box
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
cBioPortal	cBio Cancer Genomics Portal

References

[1] J. Ferlay, E. Steliarova-Foucher, J. Lortet-Tieulent, S. Rosso, J.W. Coebergh, H. Comber, D. Forman, F. Bray, Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012, Eur. J. Cancer 49 (2013) 1374–1403, https://doi.org/10.1016/j.ejca.2012.12.027.

[2] J.A. Hall, R. Salgado, T. Lively, F. Sweep, A. Schuh, A risk-management approach for effective integration of biomarkers in clinical trials: perspectives of an NCI, NCRI, and EORTC working group, Lancet Oncol. 15 (2014) e184–e193, https://doi.org/10.1016/S1470-2045(13)70607-7.

[3] T. Swedish Rectal Cancer, B. Cedermark, M. Dahlberg, B. Glimelius, L. Pahlman, L.E. Rutqvist, N. Wilking, Improved survival with preoperative radiotherapy in resectable rectal cancer, N. Engl. J. Med. 336 (1997) 980–987, https://doi.org/10.1056/NEJM199704033361402.

[4] M. Aklilu, C. Eng, The current landscape of locally advanced rectal cancer, Nat. Rev. Clin. Oncol. 8 (2011) 649–659, https://doi.org/10.1038/nrclinonc.2011.118.

[5] C. Moncharmont, A. Levy, J.B. Guy, A.T. Falk, M. Guilbert, J.C. Trone, G. Alphonse, M. Gilormini, D. Ardail, R.A. Toillon, C. Rodriguez-Lafrasse, N. Magne, Radiation-enhanced cell migration/invasion process: a review, Crit. Rev. Oncol.-Hematol. 92 (2014) 133–142, https://doi.org/10.1016/j.critrevonc.2014.05.006.

[6] S. Zona, L. Bella, M.J. Burton, G. Nestal de Moraes, E.W. Lam, FOXM1: an emerging master regulator of DNA damage response and genotoxic agent resistance, Biochim. Biophys. Acta 1839 (2014) 1316–1322, https://doi.org/10.1016/j.bbagrm.2014.09.016.

[7] G. Nestal de Moraes, L. Bella, S. Zona, M.J. Burton, E.W. Lam, Insights into a critical role of the FOXO3a-FOXM1 Axis in DNA damage response and genotoxic drug resistance, Curr. Drug Targets 17 (2016) 164–177, https://doi.org/10.2174/1389450115666141122211549.

[8] W. Weng, Y. Okugawa, S. Toden, Y. Toiyama, M. Kusunoki, A. Goel, FOXM1 and FOXQ1 are promising prognostic biomarkers and novel targets of tumor-suppressive miR-342 in human colorectal cancer, Clin. Cancer Res. 22 (2016) 4947–4957, https://doi.org/10.1158/1078-0432.CCR-16-0360.

[9] S. Zona, L. Bella, M.J. Burton, G. Nestal de Moraes, E.W. Lam, FOXM1: an emerging master regulator of DNA damage response and genotoxic agent resistance, Biochim. Biophys. Acta 1839 (2014) 1316–1322, https://doi.org/10.1016/j.bbagrm.2014.09.016.

[10] B. Lerrer, A.A. Gertler, H.Y. Cohen, The complex role of SIRT6 in carcinogenesis, Carcinogenesis 37 (2016) 108–118, https://doi.org/10.1093/carcin/bgv167.

[11] A.R. Gomes, F. Zhao, E.W. Lam, Role and regulation of the forkhead transcription factors FOXO3a and FOXM1 in carcinogenesis and drug resistance, Chin. J. Cancer 32 (2013) 365–370, https://doi.org/10.5732/cjc.012.10277.

[12] A. Lewander, J. Gao, G. Adell, H. Zhang, X.F. Sun, Expression of NF-kappaB p65 phosphorylated at serine-536 in rectal cancer with or without preoperative radiotherapy, Radiol. Oncol. 45 (2011) 279–284, https://doi.org/10.2478/v10019-011-0030-7.

[13] A. Knutsen, G. Adell, X.F. Sun, Survivin expression is an independent prognostic factor in rectal cancer patients with and without preoperative radiotherapy, Int. J. Radiat. Oncol. Biol. Phys. 60 (2004) 149–155, https://doi.org/10.1016/j.ijrobp.2004.02.007.

[14] D. Pfeifer, J. Gao, G. Adell, X.F. Sun, Expression of the p73 protein in rectal cancers with or without preoperative radiotherapy, Int. J. Radiat. Oncol. Biol. Phys. 65 (2006) 1143–1148, https://doi.org/10.1016/j.ijrobp.2006.02.028.

[15] L. Yang, J. Zhou, Q. Ma, C. Wang, K. Chen, W. Meng, Y. Yu, Z. Zhou, X. Sun, Knockdown of PPAR delta gene promotes the growth of colon cancer and reduces the sensitivity to bevacizumab in nude mice model, PLoS One 8 (2013), e60715, https://doi.org/10.1371/journal.pone.0060715.

[16] A. Holmqvist, J. Gao, G. Adell, J. Carstensen, X.F. Sun, The location of lymphangiogenesis is an independent prognostic factor in rectal cancers with or without preoperative radiotherapy, Ann. Oncol. 21 (2010) 512–517, https://doi.org/10.1093/annonc/mdp486.

[17] E. Cerami, J. Gao, U. Dogrusoz, B.E. Gross, S.O. Sumer, B.A. Aksoy, A. Jacobsen, C.J. Byrne, M.L. Heuer, E. Larsson, Y. Antipin, B. Reva, A.P. Goldberg, C. Sander, N. Schultz, The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data, Cancer Discov. 2 (2012) 401–404, https://doi.org/10.1158/2159-8290.CD-12-0095.

[18] A. Koch, J. Jeschke, W. Van Criekinge, M. van Engeland, T. De Meyer, MEXPRESS update 2019, Nucleic Acids Res. 47 (2019) W561–W565, https://doi.org/10.1093/nar/gkz445.
[19] J. Montojo, K. Zuberi, H. Rodriguez, G.D. Bader, Q. Morris, GeneMANIA: fast gene network construction and function prediction for Cytoscape, F1000Research 3 (2014) 153, https://doi.org/10.12688/f1000research.4572.1.
[20] S.V. Vasaikar, P. Straub, J. Wang, B. Zhang, LinkedOmics: analyzing multi-omics data within and across 32 cancer types, Nucleic Acids Res. 46 (2018) D956–D963, https://doi.org/10.1093/nar/gkx1090.
[21] Y. Zhou, B. Zhou, L. Pache, M. Chang, A.H. Khodabakhshi, O. Tanaseichuk, C. Benner, S.K. Chanda, Metascape provides a biologist-oriented resource for the analysis of systems-level datasets, Nat. Commun. 10 (2019) 1523, https://doi.org/10.1038/s41467-019-09234-6.
[22] C. Gong, S. Yao, A.R. Gomes, E.P. Man, H.J. Lee, G. Gong, S. Chang, S.B. Kim, K. Fujino, S.W. Kim, S.K. Park, J.W. Lee, M.H. Lee, K.s. group, U.S. Khoo, E. W. Lam, BRCA1 positively regulates FOXO3 expression by restricting FOXO3 gene methylation and epigenetic silencing through targeting EZH2 in breast cancer, Oncogenesis 5 (2016) e214, https://doi.org/10.1038/oncsis.2016.23.
[23] Y.C. Chen, C.C. Hsiao, T.W. Chen, C.C. Wu, T.Y. Chao, S.Y. Leung, H.L. Eng, C.P. Lee, T.Y. Wang, M.C. Lin, Whole genome DNA methylation analysis of active pulmonary tuberculosis disease identifies novel epigenotypes: PARP9/miR-505/RASGRP4/GNG12 gene methylation and clinical phenotypes, Int. J. Mol. Sci. 21 (2020), https://doi.org/10.3390/ijms21093180.
[24] A. Essafi, S. Fernandez de Mattos, Y.A. Hassen, I. Soeiro, G.J. Mufti, N.S. Thomas, R.H. Medema, E.W. Lam, Direct transcriptional regulation of Bim by FoxO3a mediates STI571-induced apoptosis in Bcr-Abl-expressing cells, Oncogene 24 (2005) 2317–2329, https://doi.org/10.1038/sj.onc.1208421.
[25] H. Zhu, Targeting forkhead box transcription factors FOXM1 and FOXO in leukemia (Review), Oncol. Rep. 32 (2014) 1327 1334, https://doi.org/10.3892/or.2014.3357.
[26] S. Kundu, M.A. Ali, N. Handin, N. Padhan, J. Larsson, M. Karoutsou, K. Ban, J.R. Wisniewski, P. Artursson, L. He, M. Hellstrom, T. Sjoblom, Linking FOXO3, NCOA3, and TCF7L2 to Ras pathway phenotypes through a genome-wide forward genetic screen in human colorectal cancer cells, Genome Med. 10 (2018) 2, https://doi.org/10.1186/s13073-017-0511-4.
[27] M. Kumazoe, M. Takai, J. Bae, S. Hiroi, Y. Huang, K. Takamatsu, Y. Won, M. Yamashita, S. Hidaka, S. Yamashita, S. Yamada, M. Murata, S. Tsukamoto, H. Tachibana, FOXO3 is essential for CD44 expression in pancreatic cancer cells, Oncogene 36 (2017) 2643–2654, https://doi.org/10.1038/onc.2016.426.
[28] M. Kumazoe, M. Takai, S. Hiroi, C. Takeuchi, M. Kadomatsu, T. Nojiri, H. Onda, J. Bae, Y. Huang, K. Takamatsu, S. Yamashita, K. Kangawa, H. Tachibana, The FOXO3/PGC-1beta signaling axis is essential for cancer stem cell properties of pancreatic ductal adenocarcinoma, J. Biol. Chem. 292 (2017) 10813–10823, https://doi.org/10.1074/jbc.M116.772111.
[29] S. Salcher, J. Hagenbuchner, K. Geiger, M.A. Seiter, J. Rainer, R. Kofler, M. Hermann, U. Kiechl-Kohlendorfer, M.J. Ausserlechner, P. Obexer, C10ORF10/DEPP, a transcriptional target of FOXO3, regulates ROS-sensitivity in human neuroblastoma, Mol. Cancer 13 (2014) 224, https://doi.org/10.1186/1476-4598-13-224.
[30] M. Rupp, J. Hagenbuchner, B. Rass, H. Fiegl, U. Kiechl-Kohlendorfer, P. Obexer, M.J. Ausserlechner, FOXO3-mediated chemo-protection in high-stage neuroblastoma depends on wild-type TP53 and SESN3, Oncogene 36 (2017) 6190–6203, https://doi.org/10.1038/onc.2017.288.
[31] R.C. Hui, A.R. Gomes, D. Constantinidou, J.R. Costa, C.T. Karadedou, S. Fernandez de Mattos, M.P. Wymann, J.J. Brosens, A. Schulze, E.W. Lam, The forkhead transcription factor FOXO3a increases phosphoinositide-3 kinase/Akt activity in drug-resistant leukemic cells through induction of PIK3CA expression, Mol. Cell Biol. 28 (2008) 5886–5898, https://doi.org/10.1128/MCB.01265-07.
[32] S. Abdeljaoued, L. Bettaieb, M. Nasri, O. Adouni, A. Goucha, H. Bouzaiene, H. Boussen, K. Rahal, A. Gamoudi, Forkhead box M1 (FOXM1) expression predicts disease free survival and may mediate resistance to chemotherapy and hormonotherapy in male breast cancer, Breast Dis. 37 (2018) 109–114, https://doi.org/10.3233/BD-170315.
[33] Y. Azuma, T. Yokobori, A. Mogi, B. Altan, T. Yajima, T. Kosaka, R. Onozato, E. Yamaki, T. Asao, M. Nishiyama, H. Kuwano, SIRT6 expression is associated with poor prognosis and chemosensitivity in patients with non-small cell lung cancer, J. Surg. Oncol. 112 (2015) 231–237, https://doi.org/10.1002/jso.23975.
[34] E. McCann, J. O'Sullivan, S. Marcone, Targeting cancer-cell mitochondria and metabolism to improve radiotherapy response, Transl Oncol 14 (2021), 100905, https://doi.org/10.1016/j.tranon.2020.100905.