RUNX2 and Cancer

Subjects: Cell Biology

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Runt-related transcription factor 2 (RUNX2) is critical for the modulation of chondrocyte osteoblast differentiation and hypertrophy. Recently discovered RUNX2 somatic mutations, expressional signatures of *RUNX2* in normal tissues and tumors, and the prognostic and clinical significance of RUNX2 in many types of cancer have attracted attention and led RUNX2 to be considered a biomarker for cancer. Many discoveries have illustrated the indirect and direct biological functions of RUNX2 in orchestrating cancer stemness, cancer metastasis, angiogenesis, proliferation, and chemoresistance to anticancer compounds, warranting further exploration of the associated mechanisms to support the development of a novel therapeutic strategy.

RUNX2 prognosis cancer progression

1. Introduction

In 1993, a gene family of DNA-binding transcriptional regulatory proteins was identified from *Drosophila*, mouse, and human. These genes shared a highly conserved, 128-amino-acid region characterized as a DNA-binding domain, Runt [1]. Three members, *RUNX1/Cbfa1/Pebp2αA*, *RUNX2/Cbfa2/Pebp2αB*, and *RUNX3/Cbfa3/Pebp2αC*, in this Runt-domain family were further reported in humans [2][3][4]. The human *RUNX2* (Runt-related transcription factor 2) gene was isolated from a B-cell-derived cDNA library [2]. The *RUNX2* gene is located at 6p21.1 in humans [5] and encodes various isoforms with a total of 12 transcript variants. RUNX2 is involved in osteogenesis and the maturation of chondrocytes via the modulation of transcriptional activation and multiple signaling pathways [6][7][8][9]. RUNX2 is a well-known master regulator of osteoblast and chondrocyte differentiation, but new findings have demonstrated its participation in cancer progression and tumorigenesis. Accumulated experimental data have revealed the functions of the RUNX2-mediated downstream axis in modulating angiogenesis, cancer metastasis, proliferation, cancer stemness, and drug resistance leading to cancer progression.

2. RUNX2 Expression in Cancers

RUNX2 RNA and protein expression levels in various types of cancer were measured. Relatively high RUNX2 levels were detected by IHC staining in tissues of renal cell carcinoma compared with nontumor tissue, whose regulatory mechanism required Zic family member 2 (Zic2) in 786-O and ACHN cells [10]. RUNX2 was shown to be an interactive target of miR-23a-3p in CAL-27 cells and TSCCA cells, and oral squamous cell carcinoma (OSCC) overexpressing miR-23a-3p mimics decreased the RUNX2 level [11]. RUNX2 was significantly decreased by transfection of a miRNA-218 mimic, and RUNX2 expression was obviously increased by treatment with a miRNA-218 inhibitor in osteosarcoma U2OS cells [12]. In oral cancer (both HSC-3 and Ca9-22 cells), RUNX2 expression

was positively regulated by MRE11, the nuclease component of the RAD50/MRE11/NBS1 DNA repair complex [13]. In a colorectal cancer study that enrolled 75 cancer patients, cancer tissues displayed high RUNX2 levels compared with normal adjacent tissues. Consistent results with these were obtained by Western blot analysis of 10 paired cancer and normal tissues [14]. RUNX2 protein was detected in cervical cancer tissues, and RUNX2 expression declined upon overexpression of miR-218-5p in C-33A and CaSki cells [15]. RUNX2 protein was elevated in human thyroid cancer cell lines and cancer tissues compared with primary cell lines and normal thyroid tissues [16]. RUNX2 was overexpressed in lung adenocarcinoma in a large study that included 2418 tumor and 1574 nontumor lung samples [17]. In gastric cancer, RUNX2 expression levels were analyzed by immunohistochemical staining of 60 cancer tissues and by consulting the Gene Expression Profiling Interactive Analysis (GEPIA) database, which demonstrated the high expression of RUNX2 at both the gene and protein levels in gastric cancer [18]. In oral squamous cell carcinoma (OSCC), RUNX2 RNA levels were found to be statistically higher in tumor tissues than in normal tissues by qRT-PCR analysis of 40 pathological specimens. A similar result was observed in a comparison between squamous cell carcinoma cells (TCA8113, CAL-27, SCC-9, and TSCCA) and normal oral keratinocytes (NHOK) [11]. Nickel (Ni) compounds are classified as Group 1 carcinogens, including to the lungs. RUNX2 expression appeared to be increased upon Ni-initiated BEAS-2B transformation, suggesting a potential role in lung tumorigenesis [19]. RUNX2 expression could also be orchestrated by circular RNA (circRNA)-mediated signaling. In nasopharyngeal carcinoma, circRANBP17 promoted RUNX2 expression by sponging miR-635 [20]. RUNX2 was overexpressed in tissue samples of bladder urothelial cancer, and immunohistochemistry further demonstrated the positive correlation of high RUNX2 levels with cancer-associated fibroblast biomarkers [21]. The data of integrating the transcriptomic studies in various cancer types and the matched clinical information were announced and released (University of California, Santa Cruz, *n* = 12,839) [22]. As seen in **Figure 1**, *RUNX2* was shown to be highly upregulated in pancreatic cancer, breast cancer, lung cancer, thyroid cancer, and head and neck cancer. In contrast, lower *RUNX2* levels were detected in liver cancer and testis cancer.

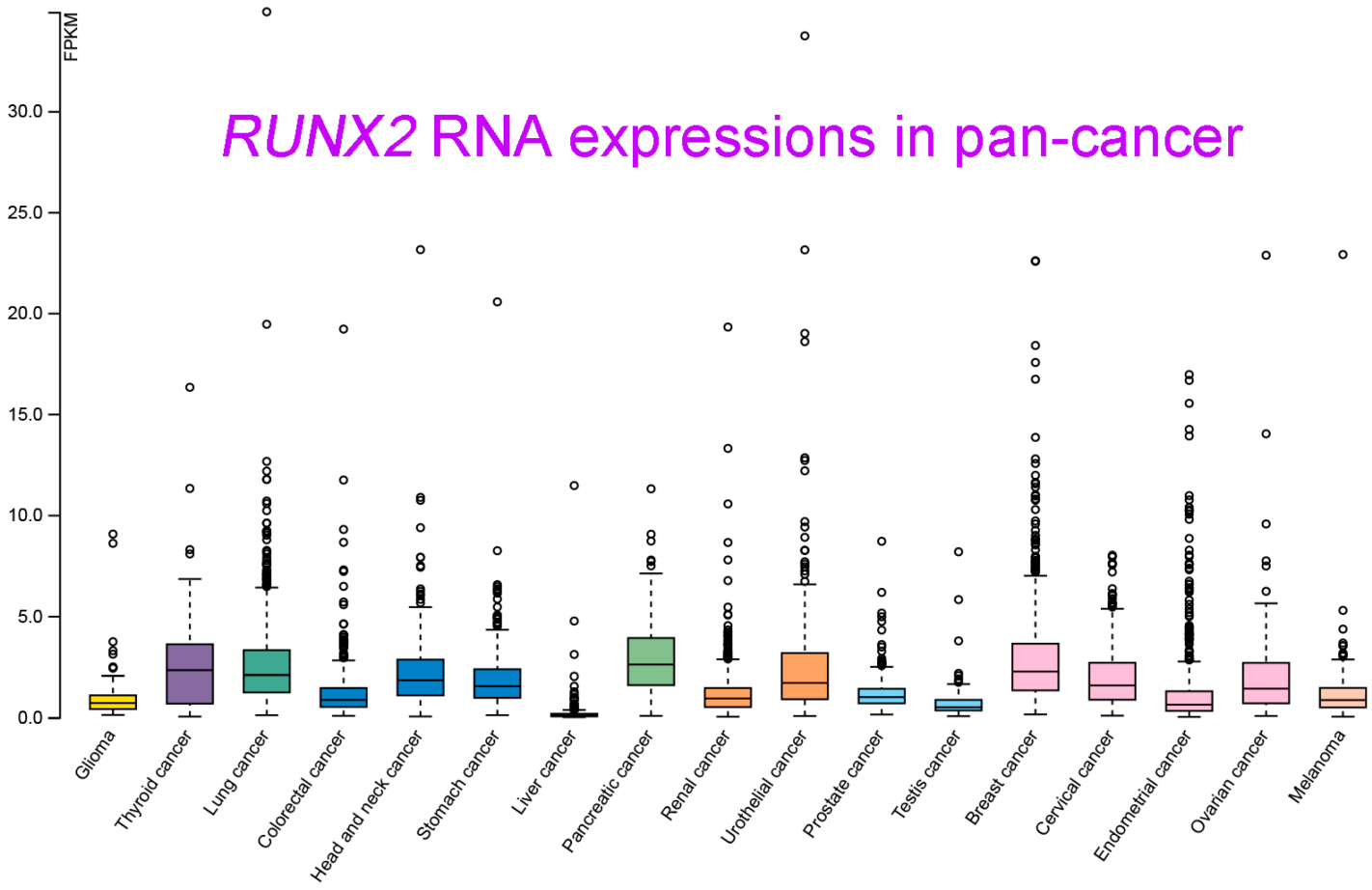

Figure 1. *RUNX2* RNA-seq data in 17 cancer types (TCGA) were re-analyzed. These transcript expression data were obtained by RNA-Seq analyses based on the data retrieved from the TCGA database and were normalized and used to assess relative RUNX2 expression in various types of cancers. Data are shown as the median number of fragments per kilobase per million (FPKM). Normal distribution in the dataset is represented by the box plots, and the points represent the data of outliers if the expression levels are below or above 1.5 times the interquartile range. Data were adapted with permission from HPA (https://www.proteinatlas.org/about/licence#citation_guidelines_for_the_human_protein_atlas, accessed on: 21 February 2023).

3. Correlation with Clinical Outcome

RUNX2 appears to be a prognostic biomarker in many cancer types. In oral cancer patients, a high RUNX2 level was correlated with lymph node metastasis [13]. Tumor budding has been characterized as a microscopic-finding-based dedifferentiation at the invasive margin in colon cancer. RUNX2 was identified as a constituent of the molecular budding gene signature and contributed to unfavorable relapse-free survival rates in a cohort study of 85 patients with stage II/III disease [23]. In an exploration of clinical data in colon cancer, RUNX2 was expressed higher in cancer patients with metastasis and shorter survival [24]. In a clinical study of gastric cancer, patients with positive RUNX2 expression had unfavorable survival, clinical stage, and associated lymph node metastasis [18]. RUNX2 expression was measured by immunohistochemistry and analyzed for correlations with clinical data in 105

osteosarcoma patients, and it appeared to be an independent predictor of metastasis-free survival and overall survival in a multivariate survival analysis. In addition, RUNX2 and osteopontin expression were strongly correlated at the protein level [25]. In lung adenocarcinoma, the expression of RUNX2 correlated with a poor hazard ratio, suggesting that RUNX2 plays a clinical role as an independent risk factor for poor survival in lung cancer [17]. A similar result demonstrated the positive correlation of elevated RUNX2 with poor overall survival of non-small-cell lung cancer patients [26]. RUNX2 expression was associated with adverse overall survival in a study of 301 renal cell carcinoma patients. In addition, correlations with poor grade and stage were revealed by an analysis of the TCGA database [10]. In hepatocellular carcinoma, the data from clinicopathological analysis of 89 samples indicated the correlation of RUNX2 expression with metastasis rate and shorter survival period [27]. An immunohistochemistry-based study of breast cancer tissue samples obtained from 75 patients showed that a high RUNX2 level was significantly associated with poor prognosis, Ki-67 expression, and lymphatic metastasis [28]. A comprehensive pancancer study integrating cancer patients' clinical data with RNA expression profiles has been completed and released from the Human Protein Atlas (HPA) [29][30][31][32][33] and Kaplan–Meier plotter [34] databases. The prognostic data of *RUNX2* in different cancer types are listed in **Table 1** (data were adapted with permission from HPA: https://www.proteinatlas.org/about/licence#citation_guidelines_for_the_human_protein_atlas, accessed on 21 February 2023). *RUNX2* appears to be an inferior prognostic biomarker in cohorts of patients with glioma, colorectal cancer, stomach cancer, pancreatic cancer, renal cancer, urothelial cancer, lung cancer, and cervical cancer. On the other hand, in patients diagnosed with breast and ovarian cancer determined by array, high *RUNX2* expression levels are correlated with better clinical outcomes.

Table 1. Correlation of *RUNX2* with cancer patient survival.

Symbol	Cancer Type	Prognosis	Endpoint	*p* Value	Case	Dataset	Method	Probe ID
RUNX2	Glioma	Poor	Overall survival	0.02	153	TCGA	RNA Seq	
RUNX2	Thyroid Cancer	-	Overall survival	N.S.	501	TCGA	RNA Seq	
RUNX2	Lung Cancer	-	Overall survival	N.S.	994	TCGA	RNA Seq	
RUNX2	Colorectal Cancer	Poor	Overall survival	0.04	597	TCGA	RNA Seq	
RUNX2	Head and Neck Cancer	-	Overall survival	N.S.	499	TCGA	RNA Seq	
RUNX2	Stomach Cancer	Poor	Overall survival	<0.001	354	TCGA	RNA Seq	

Symbol	Cancer Type	Prognosis	Endpoint	*p* Value	Case	Dataset	Method	Probe ID
RUNX2	Liver Cancer	-	Overall survival	N.S.	365	TCGA	RNA Seq	
RUNX2	Pancreatic Cancer	Poor	Overall survival	0.037	176	TCGA	RNA Seq	
RUNX2	Renal Cancer	Poor	Overall survival	<0.001	877	TCGA	RNA Seq	
RUNX2	Urothelial Cancer	Poor	Overall survival	<0.001	406	TCGA	RNA Seq	
RUNX2	Prostate Cancer	-	Overall survival	N.S.	494	TCGA	RNA Seq	
RUNX2	Testis Cancer	-	Overall survival	N.S.	134	TCGA	RNA Seq	
RUNX2	Breast Cancer	-	Overall survival	N.S.	1075	TCGA	RNA Seq	
RUNX2	Cervical Cancer	Poor	Overall survival	0.0089	291	TCGA	RNA Seq	
RUNX2	Endometrial Cancer	-	Overall survival	N.S.	541	TCGA	RNA Seq	
RUNX2	Ovarian Cancer	-	Overall survival	N.S.	373	TCGA	RNA Seq	
RUNX2	Melanoma	-	Overall survival	N.S.	102	TCGA	RNA Seq	
RUNX2	Breast Cancer	Good	Relapse-free survival	<0.001	4929	E-MTAB-365, E-TABM-43, GSE: 11,121, 12,093,	Array	216994_s_at
						12,276, 1456, 16,391, 16,446, 16,716, 17,705, 17,907,		
						18,728, 19,615,		

Symbol	Cancer Type	Prognosis	Endpoint	*p* Value	Case	Dataset	Method	Probe ID
						20,194, 20,271, 2034, 20,685, 20,711,		
						21,653, 22,093, 25,066, 2603, 26,971, 29,044, 2990,		
						31,448, 31,519, 32,646, 3494, 36,771, 37,946, 41,998,		
						42,568, 43,358, 43,365, 45,255, 4611, 46,184, 48,390,		
						50,948, 5327, 58,812, 61,304, 65,194, 6532, 69,031,		
						7390, 76,275, 78,958, 9195		
RUNX2	Ovarian Cancer	Good	Progression-free survival	0.0037	1435	GSE: 14,764, 15,622, 18,520, 19,829, 23,554, 26,193,	Array	216994_s_at

Symbol	Cancer Type	Prognosis	Endpoint	*p* Value	Case	Dataset	Method	Probe ID
						26,712, 27,651, 30,161, 3149, 51,373, 63,885, 65,986,	RNA Seq	
						9891, TCGA (N = 565)		
RUNX2	Lung Cancer	Poor	Postprogression survival	<0.001	1925	CAARRAY, GSE: 14,814, 19,188, 29,013, 30,219,	Array	216994_s_at
						31,210, 3141, 31,908, 37,745, 43,580, 4573, 50,081,	RNA Seq	
						8894, TCGA (N = 133)		
RUNX2	Gastric Cancer	Poor	Postprogression survival	<0.001	875	GSE: 14,210, 15,459, 22,377, 29,272, 51,105, 62,254	Array	216994_s_at

Survival data were collected from the Human Protein Atlas, TCGA, and Kaplan–Meier plotter databases. N.S.: no significance.

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