

Emerging roles for the RXFP1 in myeloid series leukemia

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Abstract

Purpose: The study investigates the role of Relaxin Family Peptide Receptor 1 (RXFP1) in myeloid leukemia cell function. It found that RXFP1 has been associated with cAMP, PI3K/Akt, NO/cGMP, MAPK and ERK1/2 signaling. High RXFP1 expression was associated with shorter cancer-specific survival RXFP1 mutated leukemia patients. The study suggests that RXFP1 may promote invasion and progression in both types of AML and CML.

Methods: The gene expression data were retrieved from Gene Expression Omnibus (GEO). Fold change, p.value t-test and David Functional analysis, hierarchical clustering was performed.

Conclusion: PCDH9, AREG, CTSG, RXFP1, CCDC152, ANXA, CD24 and VPRESB1 gene expression alterations were identified depending on leukemia in human monocyte cells. Seven of these genes were identified as downregulated in leukemia groups in human monocyte cells and one of these genes were identified as upregulated in leukemia cells. Therefore, it is hypothesized that downregulation or upregulation of these genes may affect AML/CML pathogenesis by reducing cell proliferation. And it is predicted that RXFP1 mutation may be an important factor for myeloid leukemia.

Keywords: AML, CML, RXFP1 mutation, Proliferation, DAVID functional

Introduction

One type of hematopoietic cancer is leukemia (LKA). The prevalence of LKA has been rising annually in recent years. The world had 18.1 million new cases of malignant tumors in 2018, of which 0.43 million (2.4%) were LKA patients. LKA incidence was rated 15th out of all malignant tumors. 300,000 (3.2%) of the 9.7 million deaths attributed to malignant tumors are caused by LKA, which has the 10th-highest mortality rate. The National Cancer Center of China produced a report on China LKA incidence and mortality from 2003 to 2007. The analysis indicated that LKA was more common than other malignancies, with an incidence ranking of 13th and a mortality rate ranking of 9th, respectively, compared to data from 1970 and 1990 (1). Acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelocytic leukemia (CML), and chronic lymphocytic leukemia make up the different forms of LKA in decreasing order of composition (2). Malignancies originating from stem cells include acute myeloid leukemia (AML)

and chronic myeloid leukemia (CML). Depending on the molecular characteristics, medication responses, disease variant and phase, and individual patient characteristics, the course and prognosis differ. Many individuals achieve long-term disease-free survival with BCR-ABL1-targeting medications for CML and intense treatment for AML (3). Nevertheless, not every patient responds to antileukemic medications, and some relapse after a predetermined amount of time. Allogeneic hematopoietic stem cell transplantation (SCT) is an option for people with disease-resistant conditions. Nevertheless, only a small group of "young" and healthy patients may be eligible for SCT. As a result, present initiatives concentrate on discovering novel targets and creating medication treatments that work better (4). Leukemic stem cells (LSCs) were proposed to improve antileukemic treatments by eliminating disease-initiating and disease-propagating cells, as well as to explain cellular and molecular hierarchies (5). Leukemic (sub)clones are arranged hierarchically according to the LSC

theory, with two types of cells: (1) more mature cells that undergo apoptosis after several cell divisions, and (2) LSCs that replace the majority of leukemic cells through their unrestricted ability to self-renew and spread leukemia (6). Within a CD34+/CD38- cell fraction, LSCs are thought to dwell in chronic-phase CML and certain AML types. However, at least some LSCs may also exist in CD34+/CD38+ subsets or occasionally even in CD34- subfractions, depending on the kind and stage of the disease. Since LSCs can spread disease, they are considered important targets for therapy, and a lot of research has been done to find molecular targets in these cells (7). Surface molecules that can be utilized to create immunotherapies that eradicate disease, like chimeric antigen receptor T-cell-based treatments, are of particular interest. Only a few numbers of clinically significant targets that are expressed exclusively on LSCs have been found, so there is still a need to find additional LSC markers that can be created as molecular targets for therapy or that may be used for diagnostic or prognostic purposes (8).

In the current study, we aimed to determine the leukemia-related altered genes, the network relations of these genes with each other and to relevant pathways using all genome profiles of leukemia cells. The results can provide important contributions to how prognosis can be affected in the presence of cancer.

Materials and methods

NGS gene expression data

The gene expression data was obtained from the Gene Expression Omnibus (GEO) database. Transcription profile data of human CD34+/CD38- stem cells and CD34+/CD38+ progenitor cells from healthy control samples versus acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) cells were obtained from GEO (GSE138883).

Processing and normalization of data

The raw data from Array Express were normalized with the Deseq2 package in the R software. Normalized transcription profile data consists of 17,624 different genes/ 17,624 probe sets. The

data contains 6 control, 4 AML and 6 CML whole genome expression data.

Variance, t test, and linear regression analysis

Among the groups, significant genes with absolute fold change value greater than 2 and p.value less than 0.05 were identified. To group the identified genes more specifically, Pearson's correlation absolute P value based on the correlation coefficient was calculated and genes above 0.05 were selected. Genes with a P value less than 0.05 and absolute fold change greater than 2 were selected by comparing the expression values of the genes with the control groups by Deseq2 package analysis. Analyses were done using GraphPad Prism 5.0 (Graphpad Prism 5 Software, San Diego, CA, USA).

Hierarchical clustering

Genes determined in linear regression analysis were hierarchically clustered with mean standardized gene expression values with the Euclidean Gene Cluster 3.0 program. The data was standardized after cluster analysis, and the standardized data were viewed using Treeview. Hierarchical clustering was performed by clustering both genes and arrays using Euclidian distance as similarity metric and complete linkage as clustering method.

Pathway enrichment analysis

To understand biological linkage behind these genes, "Database for Annotation, Visualization and Integrated Discovery" (DAVID) software was used. The pathways associated with our genes were identified.

Volcanoplot

A scatterplot that displays statistical significance (P value) vs magnitude of change (fold change) is known as a volcano plot. It makes it possible to quickly visually identify genes that have substantial statistical fold changes. These genes could be the ones with the most biological impact. In this plot we wanted to show all genes whose fc and p values are significant (Fig.1)

Plotbox

When displaying the distribution of data points over a chosen metric, box and whisker plots, sometimes referred to as box plots, are a fantastic graphic to utilize. These graphs show the ranges of the measured variables. Outliers, the median, the mean, and where most of the data points fall inside the "box" are all included in this. Graph pad prism 9.0 was used to create the plot box and the difference in expression of genes in cancer and normal cells was shown (Fig.2).

GEPIA program used for showing RXFP1 gene plot box to show the expression of selected gene in normal and leukemia groups.

Using a common processing pipeline, GEPIA is a newly created interactive web service for examining the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the TCGA and GTEx projects. Using the GEPIA program, we examined the expression of the determined genes in normal and cancerous tissue and showed this expression in a Plotbox, we created an overall survival plot based on these expressions and investigated how low or high expression of these genes affects life. Then, we compared the plotboxes obtained from the GEPIA program with the plotboxes made with the graphpad prism and we showed that the results were fully compatible, which makes our research more durable (Fig.3).

Results

stem- and progenitor cells gene expression in AML and CML cell line

According to the results, 7 genes corresponding to 7 probe sets with an absolute fold change greater than 2 and p.value less than 0.05 showed statistically significant expression alteration. For further analyses, we focused on these genes that altered expression diversity between groups. in AML and CML groups, 1 probe set was positively correlated and upregulated, and 7 probe sets were negatively correlated and downregulated.

Hierarchical cluster analysis demonstrated gene alterations between 2 certain groups: common genes between 4 AML and 6 CML groups and 6

control groups. Results defining as 7 of the probe sets were negatively correlated, highly expressed in the AML and CML groups. And conversely 1 of the probe sets were positively correlated, highly expressed in the control groups (Fig.4).

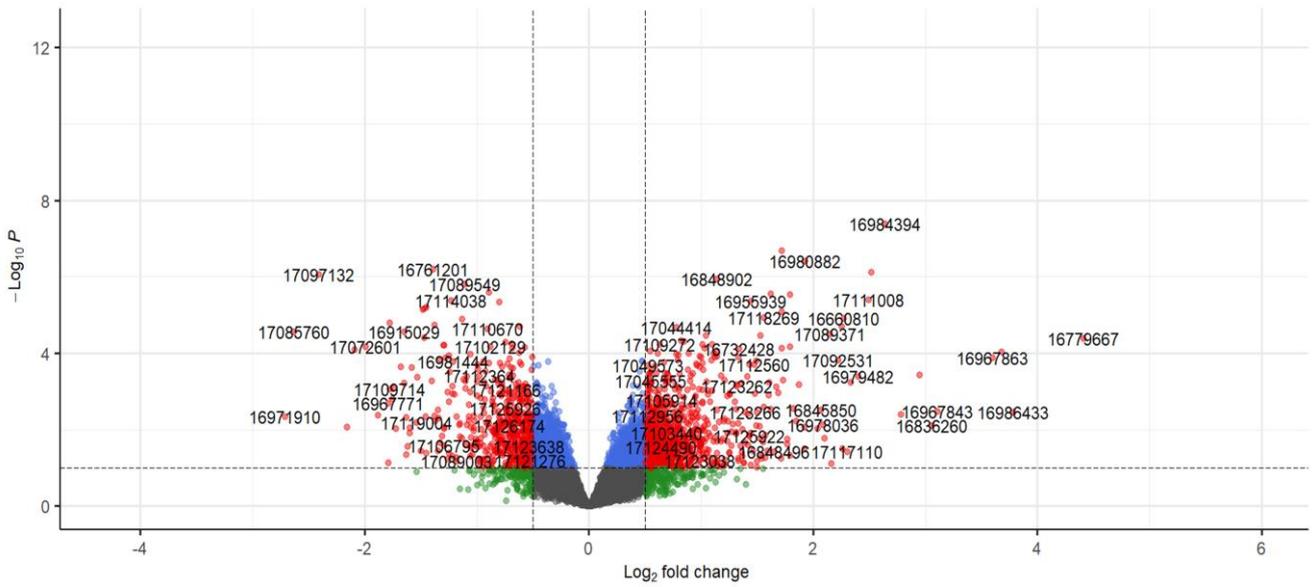


Fig. 1.A. volcano plot shows the log2 of the fold change on the x-axis and minus of the p-value. Genes with P value lesser than 0.05 and fold change greater than |3| are shown.

(Control vs AML)

Fig. 1.B. volcano plot shows the log2 of the fold change on the x-axis and minus of the p-value. Genes with P value lesser than 0.05 and fold change greater than |3| are shown.

(Control vs CML)

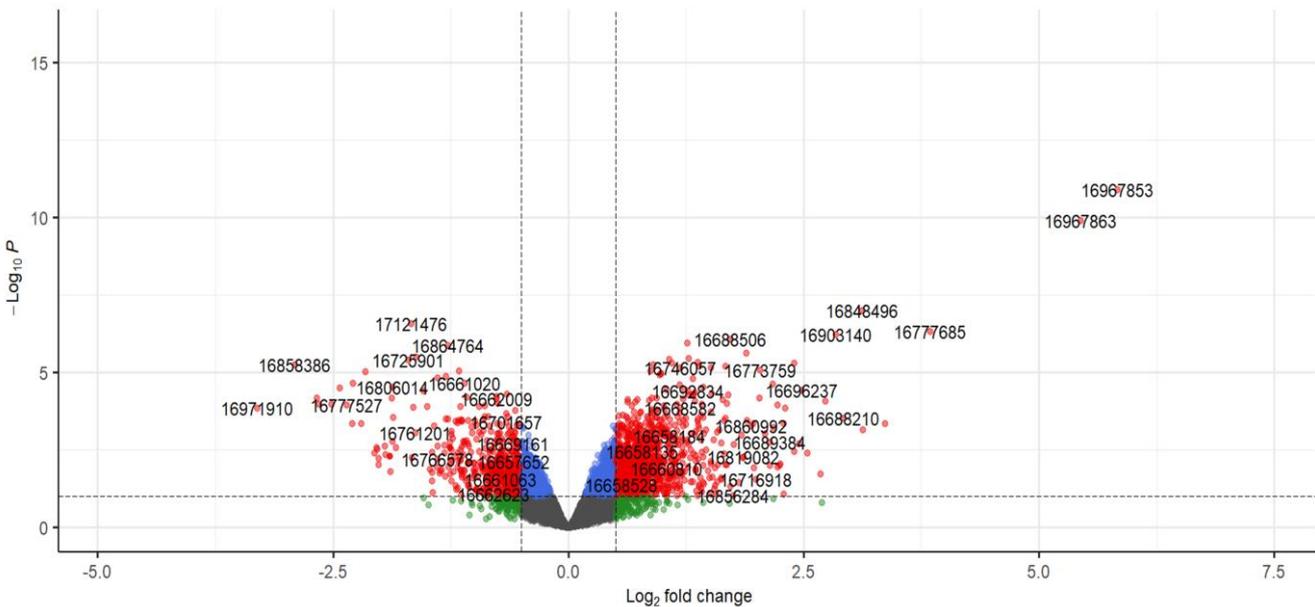


Fig.2. 7 genes of 8 genes show significant downregulated and 1 of 8 genes was significant upregulated in AML/CML groups. Statistically significant alterations were detected between myeloid leukemia groups and Control groups.

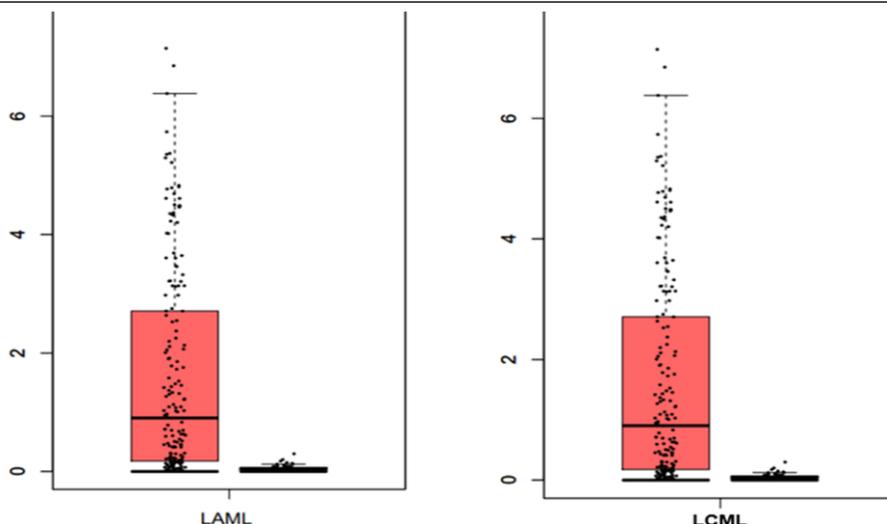
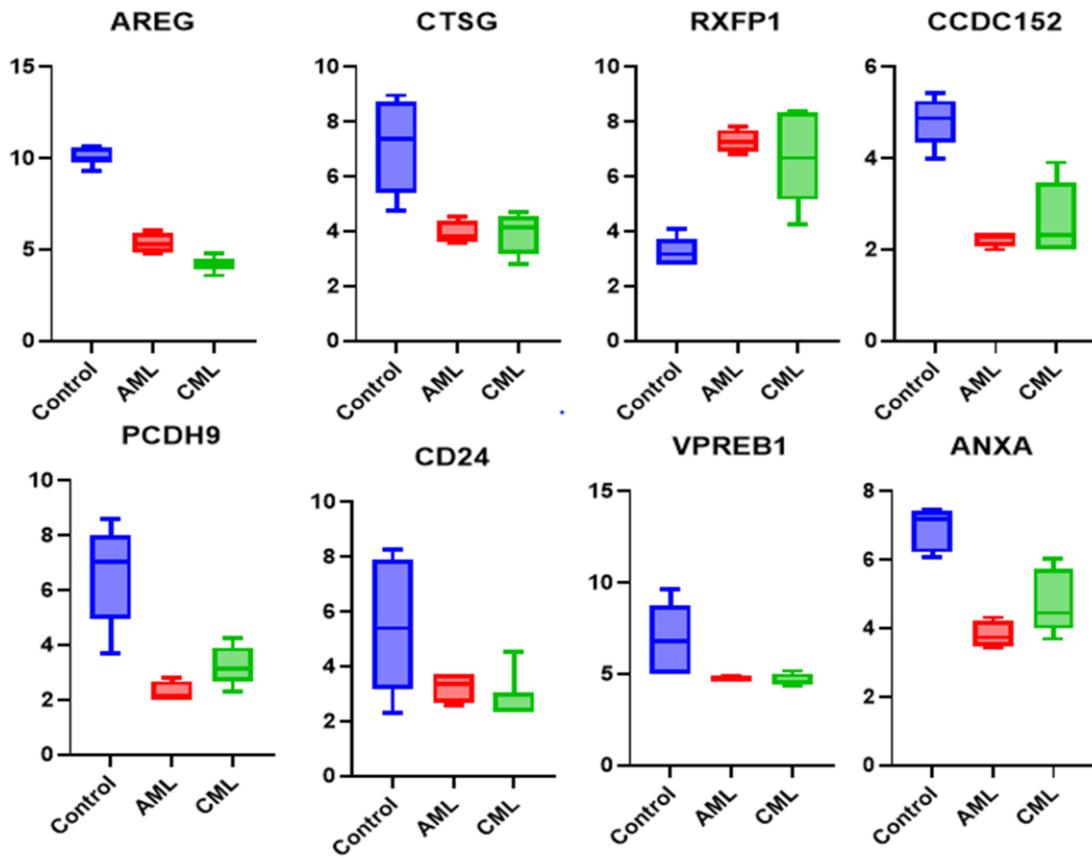


Fig.3. genes that show significant differentially expressed in patients with leukemia. Statistically significant alterations were detected between normal groups cancer group.

Gene alterations due to AML and CML

To determine whether this expression change was caused by AML/CML groups, p. Value analyses and fold change were performed between 3 groups

of 4 AML/6 CML and 6 groups of healthy cells 8 genes/8 probe set expression data.

Thus, 8 statistically significant genes (PCDH9, AREG, CTSG, RXFP1, CCDC152, ANXA,

CD24, VPREB1) corresponding 8 probe sets with an absolute fold change greater than 2 and P value less than 0.05 were determined. In linear regression analysis, 7 of 8 genes were found to be negatively correlated and downregulated in leukemia groups (Table.1).

Functional enrichment of genes and correlations with pathways

Pathway analysis of biological processes was done with DAVID software to reveal the relationship of these 8 genes with cellular functions and pathways and to understand their new meaning. Some important pathways have been identified such as MAPK signaling pathway, ErbB signaling pathway, PI3K-Akt signaling pathway, Relaxin signaling pathway, associated with 8 genes in leukemia (Table.2).

Table 1.The list of 8 genes (8 probe sets) which have the most alterations in expression. These genes have absolute fold change greater than 2 and p.value t-test value less than 0.05 between Control groups and AML/CML groups. These significant values indicate that the change occurred due to tumor.

Gene Name	Fold Change		P.value	
	AML	CML	AML	CML
PCDH9	-4.41	-3.36	4.04E-05	0.000442
AREG	-3.68	-5.83	9.28E-05	1.24E-11
CTSG	-2.78	-3.13	0.004	0.000703
RXFP1	2.71	3.31	0.00444	0.000138
CCDC152	-2.64	-2.17	4.01E-08	0.000431
ANXA	-2.4	-2.22	0.000408	0.00988
CD24	-2.3	-2.68	0.0379	0.158
VPREB1	-2.1	-2.24	3.08E-05	0.000448

Table2 Pathway analysis was done with DAVID software for 8 genes associated with cancer

Category	Pathways
KEGG_PATHWAY	regulation of cytokine-mediated signaling pathway,
KEGG_PATHWAY	positive regulation of MAP kinase activity,
KEGG_PATHWAY	Neuroactive ligand-receptor interaction,
KEGG_PATHWAY	adenylate cyclase-activating G-protein coupled receptor signaling pathway, activation of adenylylase activity, parturition,

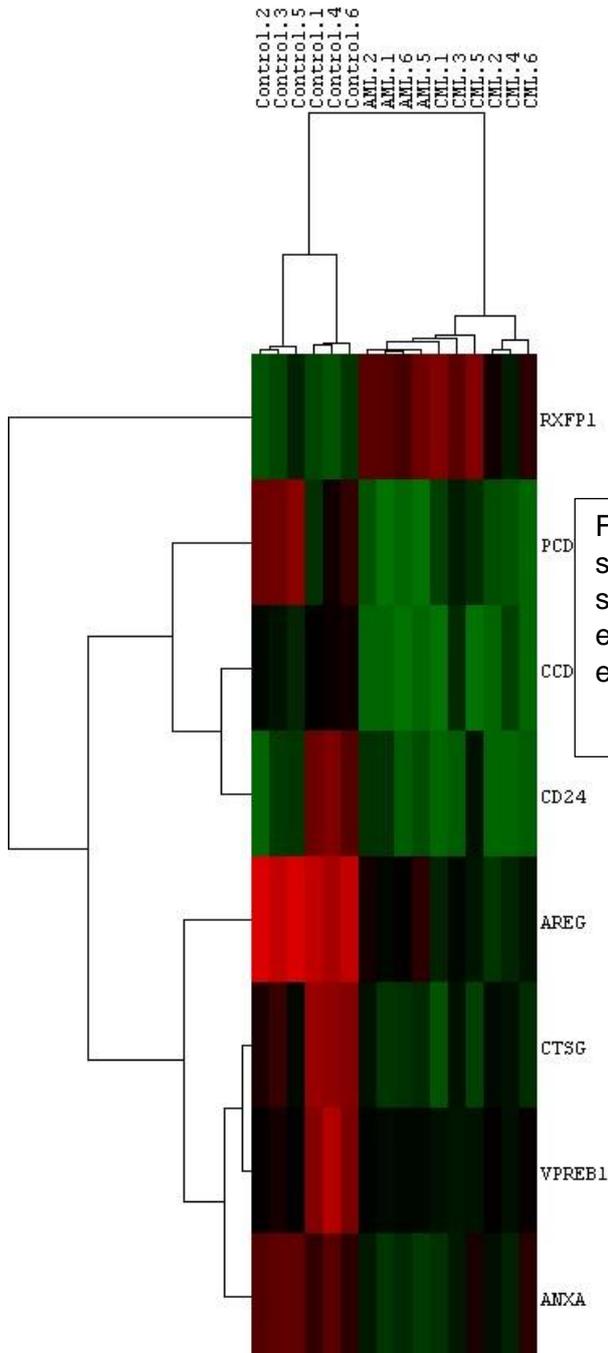


Fig.4. Hierarchical clustering of 8 statistically significant genes/8 probe sets in the three groups specified. The analysis reveals sensitive low expressions (green), intermediate (black), and high expressions (red).

Discussion

Typically, 1% to 5% of bone marrow cells are blasts, which are immature and defective cells. More than 20% blasts in the bone marrow or peripheral blood smear, which causes symptoms to appear more quickly, are the hallmark of acute leukemias. On the other hand, the start of symptoms in chronic leukemia is quite late, with less than 20% of cases having blasts. The transition from chronic myeloid leukemia to an

acute phase with a noticeably increased degree of blasts is known as the accelerated/blast phase.

PCDH9, AREG, CTSG, RXFP1, CCDC152, ANXA, CD24 and VPREB1 gene expression alterations were identified depending leukemia in human monocyte cells. Seven of these genes were identified as downregulated in leukemia groups in human monocyte cells and one of these genes were identified as upregulated in leukemia cells.

PCDHs are a subset of transmembrane proteins that are members of the superfamily cadherin.

PCDH9 caused glioma cells to enter the G0/G1 cell cycle, decreased the viability of tumor cells, and triggered apoptosis. Through activating GSK-3 β , PCDH9 prevents cell migration and the epithelial-mesenchymal transition in hepatocellular cancer (9). PCDH9 interacts with DNMT1, activated DNMT1 suppresses CDH2 and inhibit cancer metastasis (10). As a member of the epidermal growth factor (EGF) family, amphiregulin (AREG) is expressed in many different types of neoplasms, such as pancreatic, ovarian, breast, bladder, colon, and lung malignancies (11). Through the EGFR/PI3K/Akt/NF- κ B signaling pathway, AREG increased the expression of ICAM-1 and accelerated cancer cell motility in osteosarcoma. AREG triggers the NF- κ B/p65 nuclear accumulation via the EGFR/ERK signaling pathways. Through the regulation of EMT in human pancreatic cancer cells, NF- κ B facilitates the invasion and migration of cells caused by AREG. Different inhibitors were used to block these pathways, which reduced EMT in pancreatic cancer cells. For more information, see the Discussion section (12). While CTSG overexpression suppressed Akt/mTOR signaling pathways and enhanced apoptotic-associated markers, CTSG silencing stimulated Akt/mTOR signaling pathways and inhibited apoptotic-associated indicators. The Akt suppression signaling pathway of MK2206 prevents Bcl2 up-regulation and CTSG-silenced expression-induced cell survival both in vitro and in vivo (13).

Changes in many oncogenic signaling pathways, such as Src/STAT3, EGFR, HER2, Ras-like GTPase, MAPK, AKT/mTOR, WNT/ β -catenin, and miRNA-related pathways, have been associated with surface CD24 expression in tumor cells. Furthermore, it's possible that the cytoplasmic buildup of CD24 contributes to the proliferation of tumor cells, including the inactivation of p53 (14).

Signaling pathways including cAMP, PI3K/Akt, NO/cGMP, MAPK, and ERK1/2 have all been linked to the relaxin/RXFP1 system. G-proteins are recruited when relaxins bind to their receptors, which then activates adenylyl cyclase and increases cAMP. Nitric oxide (NO) and NOS2

(iNOS) expression may be enhanced by cAMP/protein kinase A-dependent NF- κ B activation. It has been demonstrated that NO inhibits profibrotic TGF β signaling by preventing Smad2 phosphorylation. Relaxin/RXFP1 can activate PI3K/Akt-associated signaling pathways to control cell differentiation and produce vasodilation in the cardiovascular system (15).

RXFP1 activates PKA/CREB/ERK1,2, activated PKA inhibits Nf-Kb that can lead to inhibits apoptosis and inflammation. Activated CREB stimulates VEGF expression and Angiogenesis.

Conclusion

Relaxin/RXFP1 signaling is important in both normal physiology and in human diseases. Research has indicated that Relaxin Family Peptide Receptor 1 (RXFP1) is predominantly expressed in progenitor cells within the blood, with minimal expression in mature myeloid cells. However, our study revealed a significant expression of RXFP1 in cells associated with both Acute Myeloid Leukemia (AML) and Chronic Myeloid Leukemia (CML). When comparing acute and chronic cases, RXFP1 expression was found to be higher in chronic cases compared to acute ones. These findings suggest that RXFP1 may serve as a marker specific to myeloid leukemia. Given that RXFP1 activation promotes anti-apoptotic mechanisms and angiogenesis through various cellular pathways, we propose that it could be a promising therapeutic target for the treatment of AML and CML.

Author contribution statement

Ayriana Safari Baesma: conception or design of the work; data acquisition; data analysis and interpretation; drafting the article; critically revising the article; final approval of the version to be published; and accountability for all aspects of the work. data acquisition and data analysis and interpretation. conception or design of the work; drafting the article; data acquisition; and data analysis and interpretation.

Berna Bayrakdar: conception or design of the work and drafting the article.

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