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Original Research Article

Annexin A1 and leukemia: A systematic review

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Abstract

Purpose: *To review systematically the involvement of Annexin A1 (ANXA1) in various leukemia cells in order to advance the understanding of ANXA1 role in leukemia.*
Methods: *The systematic review was carried out via a comprehensive search of electronic databases for all relevant articles published up to September 2017. Specific key words were used to retrieve the articles. All articles were imported into EndNote software while duplicates were removed from the list. The retrieved articles were selected using inclusion and exclusion criteria.*
Results: *FK228, a novel HDACi and FR235222, increased expression of ANXA1 in Kasumi-1, SKNO-1 and U937 cells, respectively, and induced apoptosis. The study also neutralized ANXA1 in the same cells, which caused a complete blockage of the FK228-induced apoptosis. Resveratrol was reported to markedly increase ANXA1 levels which led to caspase 3-mediated apoptosis on HL-60 cells. Dexamethasone, 17β-estradiol (E2β), all-trans retinoic acid and okadaic acid enhanced ANXA1 mRNA expression in U937, human CCRF-CEM, ATRA-NB4 and HL-60 cell lines. Rp-8-Br-cAMPs prevented dexamethasone-, E2β- and dBcAMP-induced ANXA1 synthesis via the activation of cAMP-respond-element binding protein (CREB). ANXA1 levels were reduced dramatically in K562/ADR cells as compared to K562 cells. When ANXA1 was upregulated by transfection in these cells, the cells exhibited a decrease in resistance to ADR and vincristine.*
Conclusion: *ANXA1 expression is induced by different drugs which leads to apoptosis in different types of cell. ANXA1 plays a role in the drug resistance of leukemic cells.*

Keyword: *Annexin A1, ANXA1, Lipocortin-1, Renocortin, Leukemia*

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INTRODUCTION

Leukemia is the sixth most common cancer in Malaysia, and contributes to 4.4 % of all cancer cases with 4,573 cases from the year of 2007 to 2011. Lymphoid leukemia occurs more often at younger age and its incidence increases with age [1]. Leukemia is a cancer of the early blood-forming cells, there are generally four main types of leukemia such as acute lymphocytic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, and chronic myeloid leukemia [2].

Annexin A1 (ANXA1) is reported to involve in various biological processes such as cellular transduction, proliferation, differentiation, apoptosis, inflammation and phagocytosis and these processes can relate to cancer [3]. The

ANXA1 is a 37 kDA protein and is the first characterized member of the annexin superfamily. This protein was previously recognised as renocortin, lipomodulin, lipocortin-1 and phospholipase A2 (PLA2) inhibitor [4]. It is widely distributed in the body, which includes biological fluids, lymphocytes, monocytes and granulocytes, whereby T cells and neutrophils are the predominant in the lymphocyte and granulocyte subgroups, respectively [5-7]. It possesses anti-inflammatory effects and also mimics the effects of glucocorticoids on leukocytes such as the inhibition of superoxide generation, chemotaxis and eicosanoids production [8]. ANXA1 is able to protect cancer cells when they are exposed to stress and cytotoxic agents, and thus it is said to contribute to chemotherapeutic drug resistance [9]. A study by Yu *et al* reported that ANXA1 possibly plays a role as a promising marker to predict drug resistance in bladder cancer [10]. This review reports the relationship between ANXA1 expression towards apoptosis and its contribution to drug resistance in leukemia cell lines.

Lim and Pervaiz, 2007 reported that the ANXA1 may have important regulatory roles in tumor development and progression. [4]. The protein was found to act as endogenous inhibitor of NF-κB in human cancer cells. The finding provided a novel molecular mechanism for the action of anti-inflammatory drugs and may guide for a future mechanism-driven drug to inhibit cancer development [11].

Recently, many studies that related to annexin A1 in cancer were conducted. Decreased in ANXA1 expression of ANXA1 was found to correlate with breast cancer development and progression [12]. Besides, ANXA1 was also reported to contribute to the cancer progression of oral squamous cell carcinoma and may be as a potential biomarker for the pathologic differentiation grade of this cancer [13]. This study systematically reviews the involvement of ANXA1 in various leukemia cells in order to increase the understanding of ANXA1 role in leukemia.

METHODS

Search for relevant studies

The study was conducted by using a systematic review of previous literature on ANXA1 in leukemia cell lines. The study was conducted by a comprehensive search of electronic databases namely Medline, PubMed and Scopus for all relevant articles published until September 2017. The following keywords were used for retrieval: annexin A1, annexin 1, anxa1, annexins, lipocortin 1, calpactin II, renocortin, chromobindin 9, leukemia, lymphoma, and blood cancer. Then articles were imported to EndNote software while duplicates were removed from the list.

Selection of articles

During preliminary screening titles and abstracts were evaluated. Articles that could not be categorized based on title and abstract were retrieved as full texts. The inclusion and exclusion criteria were used to screen full text articles. The inclusion criteria were for articles that reported the roles of ANXA1 in leukemia cell lines and available in English language, including those with clear description of the methodology used and results presentation. Meanwhile, review articles, editorial letters and case studies were excluded from the study (Figure 1).

Data extraction and synthesis

Data extracted included information on authors, year of study, cell line used, study findings and data of significant statistical value. A summary of the findings was presented in table form.

Bias assessment

The articles included were subjected to bias assessment by using Office of Health assessment and Translation (OHAT) risk of bias tool [14].

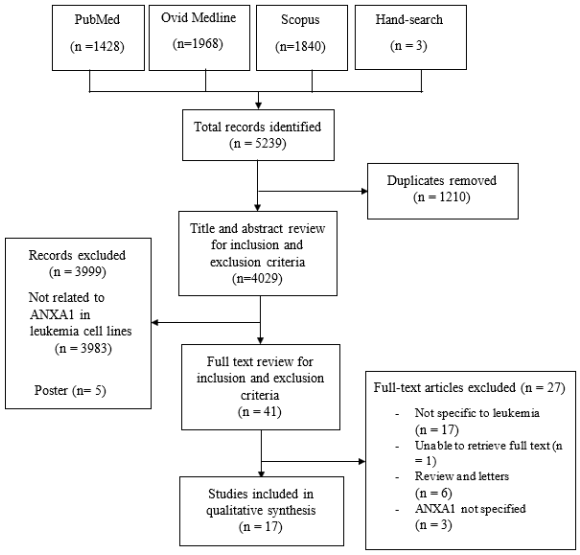

Figure 1: Flow diagram of the selection process and search strategy for systematic review of Annexin A1 (ANXA1) and Leukemia

The bias assessment included the following evaluations: attrition or exclusion bias, detection bias, selection of reporting bias that may cause

potential threats to the internal validity of the study. Based on the assessment, the study was classified into bias of low risk, high risk and unclear risk. A summary of the bias assessment risk is presented in table form.

RESULTS

ANXA1 and apoptosis

A study by Tabe *et al* investigated the effects of FK228, a novel histone deacetylase inhibitor (HDACi), in AML-eight-twenty-one oncoprotein (AML-ETO) positive cell lines, which included Kasumi-1 and SKNO-1 cells and found an upregulated ANXA1 mRNA expression. This study also showed that FK228 and SAHA, which was another type of HDACi, promoted histone acetylation in ANXA1 promoter of both types of cell tested but not in AML1-ETO-negative cells [15]. The histone deacetylase inhibitor (HDACi) was reported to be involved in apoptosis through either extrinsic or intrinsic pathway [16]. The study also showed that ANXA1 was involved in apoptosis, more than that they neutralized the ANXA1 in the same cells and caused a complete blockage of FK228-induced apoptosis [15].

A study conducted by Petrella *et al* found that the level of ANXA1 was increased by at high concentration of FR235222 in U937 cells and simultaneously apoptosis was stimulated in a time-dependent manner. In addition, it was observed that the cell death was associated with increase in levels of ANXA1 in the cytosol and the membrane of the cells. These conditions were accompanied by the appearance of 33kDa N-terminal cleavage product of ANXA1. The study also found that the effect of FR235222 on U937 cells was associated with a strong phosphorylation of ANXA1 at Ser-27 on the plasma membrane [17]. This finding is in agreement with a previous study that reported the translocation of ANXA1 to cell membrane was dependent on serine phosphorylation [18]. In a study conducted by Li *et al* on the use of resveratrol on HL-60 cells reported to markedly increase ANXA1 levels which led to caspase 3-mediated apoptosis [19]. The role of ANXA1 in apoptosis and cell proliferation was summarized in Table 1 and Table 2.

ANXA1 Induction by other compounds

A study conducted by Castro-Caldas *et al* investigated the effects of 17β-estradiol on the synthesis and secretion of ANXA1 in human CCRF-CEM cell lines. This study found that when the cells were incubated with 1μM of 17β-estradiol (*$E_2\beta$*), the ANXA1 mRNA levels were increased. However, the levels did not change with longer incubation time. This study also showed that the level of intracellular ANXA1 protein, upon incubation for 30 min, was significantly reduced to the base level. These results indicated that *$E_2\beta$* induced *de novo* synthesis of ANXA1 and stimulated its secretion in CCRF-CEM cells [20].

A study of Solito *et al* demonstrated that dexamethasone induced the expression of ANXA1 mRNA and ANXA1 protein in differentiated U-937 cells [21]. A study by Castro-Caldas *et al.* in 2002 investigated the effect of dexamethasone in CCRF-CEM cells and it was shown that dexamethasone at 1 μM decreased levels of intracellular ANXA1 protein and increased the levels of extracellular ANXA1 protein in these cells. In addition, the intracellular calcium concentration was found to increase when the cells were exposed to dexamethasone. In short, this study demonstrated that dexamethasone induced ANXA1 synthesis and calcium-dependent secretion of ANXA1 into the extracellular space [22].

A study by Tsai *et al* reported that all-trans retinoic acid (ATRA) enhanced ANXA1 expression on the cell surface of ATRA-NB4 cells in a time-dependent manner and enhanced the release of ANXA1 microparticles (MPs) from the cells when compared to controls. In addition, ANXA1 receptor (FPR2/ALX) was also increased on the surface of the cells. The study found that ANXA1 inhibited the transmigration of the cells and ATRA-NB4-derived MPs. It also mediated the anti-adhesive effect of cell-derived MPs [23]. Another study by Tsai *et al* investigated the effects of dexamethasone on the same cells. Dexamethasone enhanced the release of ANXA1-containing MPs from the cells. They found that dexamethasone inhibited the adhesion of ATRA-NB4 cells to human umbilical vein endothelial cells (HUVECS) and ANXA1 was responsible for mediating the anti-adhesive effect of ATRA-NB4-derived MPs cells [24]. This finding was consistent with another study that demonstrated that the adhesion of U937 cells to microvascular endothelium was inhibited by ANXA1 [25]. The ANXA1 induced by dexamethasone was summarized in Table 3.

A study of Sato *et al* in 1995 investigated the role of okadaic acid (OA) in HL-60 cells. It was found that OA increased the level of ANXA1 by 8-fold when as compared to the control. The level of ANXA1 mRNA was found to increase after 8h of incubation, while high levels of ANXA1 protein were observed after 16h incubation in which at the same time, phagocytosis of HL-60

Table 1: ANXA1 and apoptosis

Ref no.	Cell/tissue model cell line/ cell type	Detection technique	Finding	Comment
[15]	Kasumi-1 cell, SKNO-1 cell, NB4 cell	Oligonucleotide array-based expression profiling, real-time PCR	FK228 induced a 3.5-fold increase in the expression of ANXA1in Kasumi-1 and SKNO-1 cells and induced apoptosis this was resistance is further confirmed by upregulated ANXA1 mRNA expression by 5nM FK228.	FK228 6.4±0.2 fold compared with control.
		ChIP assay, TaqMan PCR	FK228 and SAHA promoted histone acetylation (H3L9, H4) in ANXA1 promoter in AML-ETO-positive Kasumi-1 cells and SKNO-1 cells but not AML1-ETO-negative cells.	*H3L9*; FK228 38.1±7.6-, SAHA 83.6±13.5-, *H4*; FK228 3.9±0.8-, SAHA 4.1±0.8-fold increase
		Flow cytometry	Neutralization of ANXA1 lead to a complete block of FK228-induced apoptosis.	P<0.05
[19]	HL-60	Western blot	ANXA1 levels were markedly increased after treatment with resveratrol for 12h and it was involved in caspase 3-mediated apoptosis.	$p < 0.05$

Table 2: ANXA1 and cell proliferation

Ref no.	Cell/tissue model cell line/ cell type	Detection techniques	Finding	Comment
[17]	U937 cells, K562 cells, Jurkat cells	WST-1 assay, FACS, Western blot	50nM FR235222 inhibited cell proliferation on U937 cells and this inhibition was mediated by p21 at G1 phase.	$p < 0.001$
		Western blot, quantitative PCR	ANXA1 was expressed and the ANXA1 transcripts were significantly increased by 20-fold at 24h as compared to untreated cells.	$p < 0.001$
		WST-1 assay, FACS	50nM FR235222 inhibited cell proliferation on Jurkat cells and K562cells and induced cell cycle arrest at G1 phase in Jurkat and K562 cells.	$p < 0.001$ for cell proliferation for both cells, $p < 0.01$ in Jurkat cells and $p < 0.001$ in K562 cells for induction of cell cycle arrest
		Fluorcytometric analysis, Western blot	At 0.5μM FR235222, apoptosis was stimulated in U937cells and time-dependent increased in ANXA1 expression was observed. Similar results were also observed in Jurkat and K562 cells.	$p < 0.05$

Table 3: Induction of ANXA1 by dexamethasone

Ref no.	Cell/tissue model cell line/cell type	Detection technique	Finding	Comment
[21]	U937 cells	Northern blot and Western blot	Increased ANXA1 was observed after incubation of cells with dexamethasone for more than 12h.	*p < 0.01*
[24]	NB4 cells	Flow cytometry	Dexamethasone enhanced the release of ANXA1-containing MPs from ATRA-NB4 cells.	*p < 0.05*
		Calorimetric assay	Dexamethasone inhibited the adhesion of ATRA-NB4 cells to endothelial cells (HUVECS).	*p < 0.05*
		Calorimetric assay	ANXA1 mediated the anti-adhesive effect of ATRA-NB4-derived MPs.	*p< 0.05*
[22]	CCRF-CEM cells treated with dexamethasone	Western blot and image analyzer	Dexamethasone at 1μM decreased the intracellular ANXA1 at 30 minutes and 4 hours.	*p < 0.01*
		Western blot and image analyzer	Dexamethasone at 1μM increased the extracellular ANXA1 at 4h and 12 h.	*p < 0.01*
		Perkin-Elmer LS-5B luminescence spectrometer	The intracellular calcium concentration increased when the cells were exposed to dexamethasone 1μM for 2h or 4h	*p < 0.05*

cells by phagocytes were simultaneously noted [26]. Induction of ANXA1 by 17β-estradiol, retinoic acid (ATRA) and okadoic acid (OA) was summarized in Table 4.

Mechanisms related to ANXA1

In a study by Solito *et al*, it was demonstrated that U937 cells deprived of ANXA1 showed increased phosphoplipase A_2 (PLA_2) activity in which this finding could suggest that ANXA1 played a role as endogenous inhibitor of PLA_2 [27].

A study by Castro-Caldos *et al* demonstrated that dexamethasone (dexa), $E_2\beta$ and dBCAM increased expression of ANXA1 in CCRF-CEM cells line. They also found that cells pre-treated with Rp-8-Br-cAMPs as cAMP antagonist suppressed dexa-, $E_2\beta$- and dBcAMP-induced cAMP-respond-element binding protein (CREB) and this indicated that cAMP was required for the activation of CREB. Based on the confirmation that cAMP was involved in the activation, the study found that cells pre-treated with cAMP antagonist had similar ANXA1 level as the control cells. This indicated that cAMP antagonist was effective in preventing the synthesis of ANXA1 induced by dexamethasone, $E_2\beta$ and dBcAMP. Likewise, pre-treatment with SB203580, which was a p38 MAPK inhibitor, also effectively prevented CREB activation induced by dexa, $E_2\beta$ and dBcAMP. These results indicated that both cAMP and p38 MAPK were needed for CREB activation and ANXA1 synthesis [28].

A study by Ishaq *et al* demonstrated that Zap70-negative Jurkat, P116 cells and P116/pDK (P116 cells stably inactivation Zap70 mutant) expressed reduced glucocorticoid receptor (GR)-mediated activation as compared to Jurkat T cells (wild-type) and P116/pWT (P116 cells stably Zap70 activated). This results indicated that active Zap70 was essential for normal GR-mediated activation in these cells. Besides that, this study also found that JCaM1.6 (lymphocyte-specific protein tyrosine kinase (Lck)-deficient Jurkat cells line) showed a significantly lower activity of GR as compared to JCaM1.6/vector cells (cells stably transfected with Lck), and reconstitution of Lck restored the GR activition.

These findings indicate that Lck was required in the normal GR activation in these cells. Furthermore, ANXA1 mRNA and protein levels were found to be significantly reduced in Zap70-negative and P116/pDK cells as compared to WT Jurkat cells, which indicated that the presence of Zap70 was important for normal ANXA1 expression in Jurkat T cells. The expression of ANXA1 mRNA and protein levels in P116/pWT cells was similar to that in WT Jurkat cells while in the P116/pDK cells, it expressed the similar levels with Zap70-negative cells. These indicated that Lck activity was capable of restoring normal ANXA1 expression. Since glucocorticoids were known to regulate ANXA1 levels, the study incubated the cells with dexamethasone. It was found that ANXA1 protein levels were increased in WT Jurkat cells but not in Zap70-negative cells. This finding indicated that Zap70 was important in dexa-induced ANXA1 expression [29]. The ANXA1 was found to colocalized with an integrin, $\alpha_4\beta_1$ on U937 cells induced by dexa and cause inhibition of adhesion to endothelium [25]. The mechanisms related to ANXA1 was summarized in the Table 5.

The gene member to the Ezb transformation-specific sequence family of transcription factors is called PU.1. It is expressed in monocytic, granulocytic and B-lymphoid cells [30]. In mice, PU.1-deficient exhibited failure in the development of neutrophils, macrophages and B cells [31]. A study by Iseki *et al.,* 2009 found a negative correlation between PU.1 mRNA expression and the ANXA1 mRNA expression levels in K562 cells. They suggested that decreased PU.1 and increased ANXA1 expressions might represent a poor prognostic marker for AML [31]. The Mechanisms of Zap70, Lck and PU.1 related to ANXA1 was summarized in Table 6.

Annexin A1 and drug resistance

A study by Zhu *et al* investigated the involvement of ANXA1 in multidrug resistance of chronic myeloid leukemia cell line, K562 cells. The cells were resistant to adriamycin and recognized as K562/ADR cells. The role of P-glycoprotein (P-gp) was examined in the acquired resistant cells which showed higher P-gp levels. By using normal K562 cells as a control, K562 cells showed increased accumulation of drug when compared to the resistant cells, suggesting that there was an involvement of P-glycoprotein (P-gp) in the decreased accumulation of ADR in the resistant cells. However, after one week, of drug accumulation, the resistant cells were dead without any cell dysfunction were observed while K562 cells were dead.

From the findings, the study hypothesized that upregulated P-gp was not the only factor that to acquired resistance but there were other mechanisms involved. Therefore, the study further conducted the proteome profiling of both K562 and K562/ADR cell lines and found that the

Table 4: Induction of ANXA1 by 17β-estradiol, retinoic acid (ATRA) and okadoic acid (OA)

Ref no.	Cell/tissue model cell line/cell type	Detection technique	Finding	Comment
[20]	CCRF-CEM cells	Western blot and image analyzer	ANXA1 mRNA expression increased after incubation with 1μM of $E_2β$.	$p < 0.01$
			ANXA1 mRNA expression increased with both $E_2β$ and $E_2β$ + ICI 182780.	$p < 0.05$, $p < 0.01$,
			E2β induced de novo synthesis of ANXA1 and stimulated its secretion at 30mins, 2h and 4h.	$p < 0.01$
[23]	NB4 cells	RT-PCR, Western blot	ATRA enhanced ANXA1 expression of both ANXA1 mRNA and protein levels after one day.	$p < 0.05$
		Flow cytometry	ATRA enhanced the release of ANXA1 microparticles from	$p < 0.05$
		Flow cytometry	ATRA-NB4 cells compared to controls.	$p < 0.05$
			ATRA enhanced the expression of ANXA1 receptor which is FPR2/ALX on the surface of ATRA-NB4 cells.	$p < 0.05$
			ANXA1 inhibited the transmigration of ATRA NB4 cells.	
			ANXA1 mediated the anti-migration and anti-adhesive effects of ATRA-NB4-derived MPs cells.	$p < 0.05$
[26]	HL-60	Western blot	Okadaic acid and TPA increased the amount of annexin A1 by 8- and 6-fold.	$p < 0.05$
		Northern blot	Cellular levels of ANXA1 mRNA were high after 8h of incubation and were at baseline after 24h.	
			Cellular levels of ANXA1 were high after 16h of incubation with OA and TPA.	

Table 5: Mechanisms related to ANXA1

Ref no.	Cell/tissue model cell line/cell type	Detection technique	Finding	Comments
[25]	U937 cells	Binding assay.	ANXA1 mediated anti-adhesive effects on U937 cells to endothelial cells after treatment with dexamethasone.	
		Flow cytometry, confocal microscopy.	ANXA1 co-localized with $\alpha_4\beta_1$ on U937 cells.	
[27]	U937 cells	FACS	Cells which were deprived of ANXA1 showed higher PLA_2 activity that suggested that ANXA1 might act as endogenous inhibitor of PLA_2.	$p < 0.05$
[28]	CCRF-CEM cells	Fluorescent staining	Dexamethasone, $E_2\beta$ and dBCAMP increased the expression of ANXA1 in CCRF-CEM cells as compared to control cells.	
			Cells pre-treated with Rp-8-Br-cAMPs as cAMP antagonist prevented dexa-, $E_2\beta$- and dBcAMP-induced cAMP-respond-element binding protein (CREB)	
			Pre-treatment with SB203580, which was a p38 MAPK inhibitor, effectively prevented CREB activation induced by dexa, $E_2\beta$ and dBcAMP.	

Table 6: Mechanisms of Zap70, Lck and PU.1 related to ANXA1

Ref no.	Cell/tissue model cell line/cell type	Detection technique	Finding	Comment
[29]	Jurkat T cell	Transfection and RNAi, Gene array, Western blot.	P116 Zap70-negative cells and P116/pDK (P116 cells stably inactivation Zap70 mutant) expressed reduction in glucocorticoid receptor (GR)-mediated activation as compared to Jurkat T cells (wild-type) and P116/pWT (P116 cells stably Zap70 activated). JCaM1.6 (Lck-deficient Jurkat cells) showed a significantly lower of GR activity as compared to JCaM1.6/vector cells (JCaM1.6 stably transfected with Lck), and reconstitution of Lck restored the GR activation. ANXA1 mRNA levels and ANXA1 protein were found to be reduced significantly in P116 Zap70-negative and P116/pDK cells when compared to WT Jurkat cells. The expression of ANXA1 mRNA and protein levels in the P116/pWT cells was similar with WT Jurkat cells while the P116/pDK cells expressed the ANXA1 mRNA and protein levels similar with P116 Zap70-negative cells and these indicated that protein of tyrosine kinase activity was capable of restoring normal ANXA1 expression in Jurkat cells. ANXA1 protein levels increased in WT Jurkat cells but not in P116 Zap70-negative cells after induced by dexamethasone.	
[31]	AML cells	PCR amplification	There was a negative correlation between PU.1 mRNA expression levels and the ANXA1 mRNA expression levels.	$R = -0.31$, $p < 0.042$

ANXA1 levels were dramatically reduced in resistant cells when compared to K562 cells. When ANXA1 was upregulated by transfection in K562/ADR cells, the cells exhibited a decrease in resistance to ADR and vincristine while ANXA1 knocked down K562 cells, showing an enhanced resistance towards these drugs.

When DNA fragmentation was observed in the transfected K562/ADR cells with ANXA1, it was found to be stronger than in normal K562/ADR cells, which possibly indicated that upregulated ANXA1 might resensitize K562/ADR cells via increased in apoptosis. Therefore, the authors suggested that ANXA1 might play a role in determining the response of K562 cells towards chemotherapy [32].

A study conducted by Wu *et al* demonstrated that dexamethasone increased the levels of ANXA1 in U937 cells and if TNFα was used to treat the cells, ANXA1 levels were further enhanced. Then ANXA1 mRNA levels were upregulated by dexamethasone in a time-dependent manner. Considering both findings, the study investigated the relationship between ANXA1 and TNFα-mediated apoptosis. It showed that cells primed with dexamethasone in the presence of ANXA1 antisense oligonucleotide remained sensitive to TNFα. When a nonsense oligonucleotide of ANXA1 was used, the cells developed resistance. Following the findings, the study assessed ANXA1 levels in 12 blast populations from AML patients, in which the cells were found to be resistant to TNFα and these cells as compared to TNFα-sensitive U937 and HL60 cells. The results showed that 11 out of 12 AML blast populations had cytoplasmic ANXA1 levels in excess as compared to U937 and HL60 cells. In summary, the results suggested that high levels of ANXA1 might contribute to the mechanism of resistance towards TNFα-mediated killing [33].

A study by Chen *et al* investigated the roles of ANXA1 in glucocorticoid signaling in Human T-cell leukemia virus type I (HTLV-I)-transformed. They found that interleukin-2-independent ED40515 T cells (I-ED T cells) were resistant to dexa in both cell growth and apoptosis. They then further investigated further the expression of ANXA1 mRNA and protein levels which were constitutively high in four separate IL-2 independent T cell lines which included the I-ED T cell, Adult T cell leukemia (ATL)43, ATL2, ATL35. Meanwhile, in IL-2 dependent T cell lines, ANXA1 expression was negligible. When ANXA1 was knocked down with siRNA in I-ED T cells, the cells showed cell growth retardation and enhanced cell apoptosis. Therefore, these results suggested that high expression of ANXA1 contributed to the promotion of cell proliferation in I-ED T cells, which represented an advanced stage of leukemogenesis during which the T cells were multi-resistant to apoptosis induced by chemotherapies, including Glucocorticoids (GC) [34]. ANXA1 related with drug resistance was summarized in Table 7 and Table 8.

Bias assessment

The findings showed that the majority of the articles were with low risk of bias. Only a few had high risk of detection and reporting bias. This may include that the detection procedure was not explained well and/or the results might report on selected findings.

DISCUSSION

Histone deacetylases are enzymes that have crucial roles in several biological processes through their repressive actions on transcription process [35]. It was previously reported that treatment of tumor cells with HDACi caused histone acetylation, and thus leading to repression of gene expression that increased the susceptibility of cells towards apoptosis. The HDACi induced apoptosis was through either extrinsic or intrinsic pathway [16]. Both FK228, a depsipeptide and FR235222, a cyclopeptide are HDAC inhibitors that were found to increase ANXA1 and led to the apoptosis in U937 cell. The compounds also inhibited cell proliferation and induced cell cycle arrest at G1 phase in Jurkat and K562 cell lines. These findings were important as the compounds might be used in the treatment for specific target leukemia cells and advance condition of leukemia [15,17].

The use of retinoic acid in acute promyelocytic leukemia (APL) was first described by Flynn et al in a patient with APL refractory to chemotherapy. It was found that the use of 13-cis-retinoic acid increased the number of peripheral white blood cell count and the maturing myeloid cell count [36]. Huang and colleagues in 1988 then conducted a study in 24 patients with APL by using all-trans retinoic acid (ATRA) and suggested that ATRA was an effective compound for attaining a complete remission in APL patients [37]. The study by Tsai *et al* has demonstrated that ATRA enhanced ANXA1 expression on the cell surface of ATRA-NB4 cells, released ANXA1 MPs from ATRA-NB4 cells and increased the number of FPR2/ALX on the surface of ATRA-NB4 cells.

Annexin A1 inhibited the transmigration of ATRA-NB4 cells and ATRA-NB4-derived MPs. It also

Table 7: ANXA1 related with adriamycin (ADR) and vincristine resistance

Ref no.	Cell/tissue model cell line/cell type	Detection technique	Finding	Comment
[32]	K562 cells	Western blotting, MTT assay UV visualization	ANXA1 upregulated in K562/ADR cells exhibited a decrease in resistance to both adriamycin and vincristine while there was an enhanced resistance in ANXA1 knocked down K562 cells towards adriamycin and vincristine. DNA fragmentation was found to be stronger in ANXA1 transfected K562/ADR cells as compared to normal K562/ADR cells and this suggested that upregulated ANXA1 expression resensitized the cells to adriamycin via increased in apoptosis.	$p < 0.05$

Table 8: ANXA1 related with dexamethasone resistance

Ref no.	Cell/tissue model cell line/cell type	Detection technique	Finding	Comment
[33]	U937 cell line	Western blot	Dexamethasone enhanced the expression of ANXA1 after 48h incubation.	
		Western blot	The ANXA1 expression was further enhanced by treatment with TNFα following exposure to dexamethasone.	
		RT-PCR	Dexamethasone upregulated ANXA1 mRNA expression in a time-dependent manner.	*P < 0.01*
		Mean Fluorescence Intensity.	Cells primed with dexamethasone in presence of ANXA1 antisense oligonucleotide were sensitive to TNFα when a ANXA1 nonsense oligonucleotide was used, the cells developed resistance.	
			11 out of 12 AML blast populations had cytoplasmic ANXA1 levels in excess of those seen in U937cells and HL-60 cells.	
[34]	ED40515 T-cell line	ELISA BrdU (colorimetric kit), Annexin-V FITC staining.	I-ED T-cells were resistant to Glucocorticoid (GC) in both cell growth and apoptosis.	
		Semi-quantitative RT-PCR, Quantitative RT-PCR, Immunoblotting.	ANXA1 at mRNA and protein levels were constitutively high in four separate IL-2 independent T cell lines that include I-ED T cell, ATL43, ATL2, ATL35). Meanwhile, in IL-2 dependent T cell lines, ANXA1 expression is negligible.	*p < 0.001* for ATL43, ATL2, ATL35
		Immunoblotting.	I-ED T cells knocked down of ANXA1 by siRNA showed cell growth retardation and enhanced cell apoptosis.	*p < 0.05 at 24h, p < 0.01 at 48h*

mediated the anti-adhesive effect of ATRA-NB4-derived MPs [24]. Then, Tsai *et al* investigated the effects of dexamethasone on ATRA-treated NB4 cells.

The release of ANXA1-containing MPs was enhanced and it mediated the anti-adhesive effects, which was one of the anti-inflammatory effects of ATRA-NB4-derived MPs [23]. The study by Solito *et al* has also demonstrated the anti-adhesive effects of ANXA1 [25]. These findings were consistent with a previous study which reported that ANXA1 played a role in mediating part of the anti-inflammatory effect of glucocorticoids [38].

Okadaic acid is a phosphatase inhibitor which has high specificity to type I and type IIA phosphatases [39]. In the study, it was reported that okadaic acid rapidly stimulated phosphorylation of protein in intact cells and played a role as a specific protein phosphatase inhibitor in metabolic processes [40]. The study of Sato *et al* was conducted to identify the role of OA in HL-60 cells. It was found that OA could increase ANXA1 and ANXA1 mRNA after incubation of the cells with OA. However, in this study, when the levels of ANXA1 and its mRNA were increased, the adhesion of HL60 cells was induced and phagocytosis was noted [26].

The mechanism of induced ANXA1 was described in three studies, which were the study by Castro-Caldos *et al* who found cAMP that was involved in the dexamethasone- and $E_2\beta$-induced increased expression of ANXA1 [28]. The Zap-70 was a cytoplasmic protein tyrosine kinase involved in regulating the initiation of T-cell responses by antigen receptor [41]. A study by Ishaq *et al* demonstrated that ANXA1 was significantly reduced in Zap70-negative and Zap70-inactive Jurkat cells and the transcriptional analysis showed that dexamethasone-inducible GR-mediated ANXA1 promoter activation was compromised when active Zap70 was absent [29].

When dexamethasone was used to incubate U937 cells, the ANXA1 colocalized with $\alpha_4\beta_1$ and caused anti-adhesive effects [25]. Therefore, from the studies found that Zap70 and the colocalization of $\alpha_4\beta_1$ were required for ANXA1 to mediate the effects of dexamethasone. Previously, it was reported that low PU.1 expression in APL patients caused APL initiation and progression [42]. A study by Iseki *et al* reported that in AML cells, PU.1 mRNA levels negatively correlated with ANXA1 level. The authors suggested that decreased PU.1 and increased ANXA1 expression might represent a poor prognostic marker for AML or other types of leukemia [31].

Apoptotic cells are known to release anti-inflammatory mediators, such as ANXA1 and lactoferrin [43]. The cells that undergo apoptosis will release ANXA1 and promote efferocytosis [44,45]. A study by Wu *et al* has reported that U937 cells primed with dexamethasone when stimulated with TNFα had increased the expression of ANXA1. High levels of ANXA1 contributed to the mechanism of resistance towards TNFα-mediated apoptosis via negative-feedback loop mechanism that protected the cells against their own actions [33].

Moreover, Chen *et al* reported that high expression of ANXA1 induced by glucocorticoid might contribute to the promotion of cell proliferation that represented an advanced stage of leukemogenesis in I-ED T cells multi-resistant to apoptosis induced by chemotherapies [34]. From these studies, the outcomes differed based on the types of cell line used which might have different characteristics.

CONCLUDING REMARKS

The review has identified studies that are important for understanding the roles of ANXA1 in leukemic cells. Studies reported that increase in ANXA1 expression was caused by FK228, which is a novel HDACi and FR235222, and resveratrol could induce apoptosis. There are many different mechanisms involved in the induction of ANXA1 expression, such as cAMP, CREB activation, Zap70 signalling and also PU.1 gene presence. Annexin A1 expression was also induced by many agents, such as dexamethasone, 17β-estradiol, okadaic acid (OA), and all-trans retinoic acids (ATRA).

Dexamethasone, which increases ANXA1 levels in U937 cells, participated in the resistance towards TNFα-mediated apoptosis. In I-ED cells that represents an advanced stage of leukomogenesis in which T cells, are multi-resistant to chemotherapeutic drugs, dexamethasone increased ANXA1, and led to increased cell proliferation. However, in another study that utilized K562 cells, reduced ANXA1 levels were found to associate with chemotherapeutic (adriamycin and vincristine) resistance. The different cell types showed different responses to the ANXA1 expression in developing drug resistance.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this study.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. EK developed the idea to review the topic and coordinated the research activities. PGYH performed the systematic review, EK and EMH carried out and organized the finding. The manuscript was written by PGYH and EMH and reviewed by EK. All authors read and approved the manuscript.

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