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Curcumin regulates signal transducer and activator of transcription (STAT) expression in K562 cells

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STAT, signal transducer and activator of transcription
IFN, interferon
IL, interleukin
ISRE, interferon-stimulated response element
Jak, Janus kinase
FCS, foetal calf serum

ABSTRACT

Signal transducers and activators of transcription (STATs) play important roles in numerous cellular events as for example differentiation, inflammation or immune response. Furthermore, constitutive STAT activation can be observed in a high number of tumors. In our hands, curcumin treatment induced a decrease of nuclear STAT3, -5a and -5b, without affecting neither STAT1, nor the phosphorylation state of STAT1, -3 or -5 in the K562 cell line. Most interestingly, the decrease of nuclear STAT5a and -5b after curcumin treatment was accompanied by an increase of truncated STAT5 isoforms, indicating that curcumin is able to induce the cleavage of STAT5 into its dominant negative variants lacking the STAT5 C-terminal region. Interferon (IFN)-β and -γ treatment induced IFN-stimulated responsive element (ISRE) transcriptional activity, which was efficiently inhibited by curcumin pre-treatment. In parallel, IFN-γ treatment induced an increase of the amount of nuclear STAT1 and -3, as well as their phosphorylated isoforms. Again, curcumin pre-treatment inhibited these increases. Finally, curcumin treatment inhibited Jak2 mRNA expression as well as cyclin D1 and v-src gene expression in K562 chronic leukaemia cells.

1. Introduction

STAT (signal transducers and activators of transcription) proteins are a family of transcription factors activated by extra-cellular signaling proteins such as cytokines, hormones and growth factors [1–6]. As signal transducers, STAT molecules play a role in differentiation, cell growth, apoptosis, inflammation, immune response and many other cellular events. Due to the very diverse biologic processes influenced by STAT proteins, a deregulation or loss of STAT expression can lead to severe cellular damages, or even to embryonic lethality for STAT3 knockout mice [7].

Previous studies demonstrated that tumorigenesis is often associated with constitutive activation of STATs [6,8]. In chronic myeloid leukaemia, characterized by the presence of the Philadelphia chromosome generating the Bcr-Abl gene, persistent tyrosine kinase activity leads to constitutive activation of STAT proteins. The pattern of STAT activity however is cell-lineage dependent, leading to a differential STAT activation depending on the cell lined studied.

Due to the constitutive activation of STAT molecules in cancer cells and their numerous implications in the cellular events, the study of signaling cascades controlling the STATs as well as the evaluation of the mode of action displayed by

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potential anti-cancer drugs become more and more important in order to detect new potential targets and strategies to improve cancer therapy.

Curcumin is a chemopreventive agent given in clinical trials to patients with early stages of cancer [9], or allowing to sensitise ovarian cancer cells to cisplatin, thus enhancing the chemotherapeutic treatment [10]. This molecule is known to induce apoptosis in various cell lines [11], presents anti-inflammatory effects by inhibiting cyclooxygenase 2 and 5-lipoxygenase [12] and inhibits the activation of transcription factors such as nuclear factor kappa B (NF-κB) or activating protein 1 (AP-1) [13,14]. The action of curcumin on the STAT proteins largely remains to be elucidated. It was reported that curcumin inhibits oncostatin M stimulated STAT1 phosphorylation and its DNA-binding activity, without affecting p38 phosphorylation [15]. Furthermore, curcumin is able to inhibit IL-12-induced tyrosine kinase phosphorylation of STAT3 and STAT4 in activated T cells [16], as well as inhibiting the activated phosphorylation of STAT1 and STAT3 in microglia cells [17]. Finally, this chemopreventive agent inhibits IFN-α-induced STAT1 phosphorylation, as well as IL-6-induced phosphorylation and consequent STAT3 nuclear translocation in multiple myeloma cells, without affecting STAT5 phosphorylation [18,19].

Whereas all these studies focus on the action of curcumin on induced STAT proteins, the action of curcumin on constitutive STAT was only partially described for STAT3 in primary effusion lymphoma [20]. In the present report, we investigated the effect of curcumin treatment on the nuclear expression of STAT1, -3, -5a and -5b in K562 chronic myelogenous leukaemia cell with or without IFN-β and -γ induction.

2. Materials and methods

2.1. Cell culture

K562 (human chronic myelogenous leukaemia, purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen) cells were cultured in RPMI medium (Bio-Whittaker) containing 10% (v/v) foetal calf serum (FCS, Bio-Whittaker) and 1% (v/v) antibiotic–antimycotic (Bio-Whittaker) at 37 °C and 5% (w/w) of CO_2.

2.2. Reagents and antibodies

Curcumin was purchased from Sigma and dissolved in 100% DMSO at 20 mM. IFN-β and IFN-γ were purchased from Sigma. Subsequent dilutions of curcumin or IFNs were performed in cell culture medium. Western blot antibodies for the detection of STAT1 (E23), STAT3 (K15), STAT5a (L20), STAT5b (G2) and the N-terminal part of STAT5a and -5b (N20) were purchased from Santa-Cruz Biotechnology. Phosphospecific antibodies detecting p-STAT1 (Tyr701), p-STAT3 (Tyr705) and p-STAT5 (Tyr694) were from Cell Signaling Technologies. A monoclonal mouse anti-β-actin (Sigma) was used as loading control.

2.3. Nuclear and cytoplasmic protein extraction

After treatment, cells were harvested and washed. Extracts were prepared from K562 cells according to Muller et al. [21]. Protein content was determined for each sample using the Bradford assay (BioRad).

2.4. Western blot

Ten micrograms nuclear proteins or 20 μg cytoplasmic proteins were run on a 4% acrylamide concentration gel and a 10% acrylamide separation gel and transferred on a Hybond P membrane (AP-Biotech). Results were acquired on photography films as well as a Kodak 440 chemoluminescence reader and quantified by Kodak 1D software (Analis).

2.5. Transient transfections

Transfections of K562 cells were performed by electroporation using a BioRad gene pulser. For each experiment 3.75×10^6 cells at a concentration of 1.5×10^7 cells/mL were electroporated at the following settings: 250 V and 500 μF. Twenty micrograms of luciferase reporter plasmid and 5 μg of

Table 1 – Sequences used for MultiGene-12™ RT-PCR Profiling

Gene name	UniGene no.	GenBank accession no.
JAK1	Hs.436004	NM_002227
JAK2	Hs.434374	NM_004972
JAK3	Hs.210387	NM_000215
Stat1	Hs.21486	NM_007315
ISGF-3	Hs.72988	NM_005419
Stat3	Hs.421342	NM_003150
STAT4	Hs.80642	NM_003151
MGF	Hs.437058	NM_003152
STAT5	Hs.434992	NM_012448
STAT6	Hs.437475	NM_003153
Tyk2	Hs.75516	NM_003331
FIGF	Hs.11392	NM_004469
CCND1	Hs.523852	NM_053056
IL20	Hs.272373	NM_018724
IL31RA	Hs.55378	NM_139017
NMI	Hs.54483	NM_004688
OSM	Hs.248156	NM_020530
PPP2R1A	Hs.467192	NM_014225
PRLR	Hs.368587	NM_000949
SOCS1	Hs.50640	NM_003745
SOCS6	Hs.44439	NM_004232
SRC	Hs.195659	NM_005417
GAPDH	Hs.544577	NM_002046

Abbreviations: JAK1, Janus kinase 1; JAK2, Janus kinase 2; JAK3, Janus kinase 3; Stat1, signal transducer and activator of transcription 1; ISGF-3, interferon-stimulated gene factor-3 (Stat2, signal transducer and activator of transcription 2); Stat3, signal transducer and activator of transcription 3; STAT4, signal transducer and activator of transcription 4; MGF, mammary gland factor (Stat5A, signal transducer and activator of transcription 5A); STAT5, signal transducer and activator of transcription 5; STAT6, signal transducer and activator of transcription 6; Tyk2, tyrosine kinase 2. FIGF, c-fos induced growth factor (VEGFD, vascular endothelial growth factor D); CCND1: cyclin D1; IL20, interleukin 20; IL31RA, interleukin 31 receptor A; NMI, N-myc (and STAT) interactor; OSM, oncostatin M; PPP2R1A, protein phosphatase 2; PRLR, prolactin receptor; SOCS1, suppressor of cytokine signaling 1; SOCS6, suppressor of cytokine signaling 6; SRC, V-src sarcoma; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

phRG-TK-Renilla plasmid (Promega) were used for each pulse. After 24 h the cells were harvested and resuspended in growth medium (RPMI/FCS 0.1%) to a final concentration of 10^5 cells/mL in wells of microtitration plates and then treated or not. Following treatments, 75 μL Dual-Glo™ Luciferase Reagent were added to cells for a 10-min incubation at 22 °C before luciferase activity assay. Then 75 μL Dual-Glo™ Stop & Glo® Reagent were added for 10 min at 22 °C in order to assay Renilla activity. Firefly and Renilla luciferase activities were measured using a Berthold Orion microplate luminometer by integrating peaks of light emission for 10 s. Data are relative values of firefly luciferase normalised to Renilla luciferase.

2.6. Analysis of gene expression by PCR

cDNA synthesis was realized using the SuperScriptTM First-strand Synthesis System for RT-PCR (Invitrogen). Briefly, 5 μg of total RNA, extracted from K562 cells with or without treatment with curcumin at 20 μM for indicated times, were submitted to reverse transcription (RT) using oligo(dt) primers. The resulting RT products were used as templates for PCR amplification using MultiGene-12™ RT-PCR Profiling Kit (Superarray Bioscience Corp) according to the manufacturer's instructions (Table 1). The amount of cDNA synthesized was evaluated by amplification of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. PCR products were separated on a 2% agarose gel. Hundred base pairs of molecular size marker (Promega) was used for the identification of the size of the amplified fragments.

3. Results

3.1. Curcumin reduces nuclear expression of STAT3, -5a and -5b

In order to determine the effect of curcumin on STAT expression in K562 cells, nuclear extracts of untreated or curcumin treated cells were subjected to Western blot analysis. The use of a specific STAT1 antibody raised against the C-terminus of STAT1 shows that this transcription factor is constitutively present in the nucleus of K562 cells. Furthermore, Western blot analysis displays no variation of this protein after a 20 μM curcumin treatment (Fig. 1A) between 2 and 48 h. STAT3 expression, analysed with a specific antibody raised against an internal region of this protein (Fig. 1B), shows a significant decrease of nuclear STAT3 expression after 24 h of curcumin treatment, with the strongest reduction (40%) between 40 and 48 h. An antibody directed specifically against the C-terminus of STAT5a displays a significant decrease (18%) of this protein in Western blot analysis after 32 h of treatment. The strongest decrease (58%) can be observed after 48 h of treatment. Finally, STAT5b nuclear expression was analysed with an antibody targeting the STAT5b C-terminal region. Again, a significant decrease (32%) of the nuclear expression of this protein can be observed after 32 h of treatment, with a maximum (39%) decrease after 48 h of treatment. β-Actin was used as loading control for all experiments.

In order to investigate the critical curcumin concentration needed to induce a variation in STAT expression, Western blot

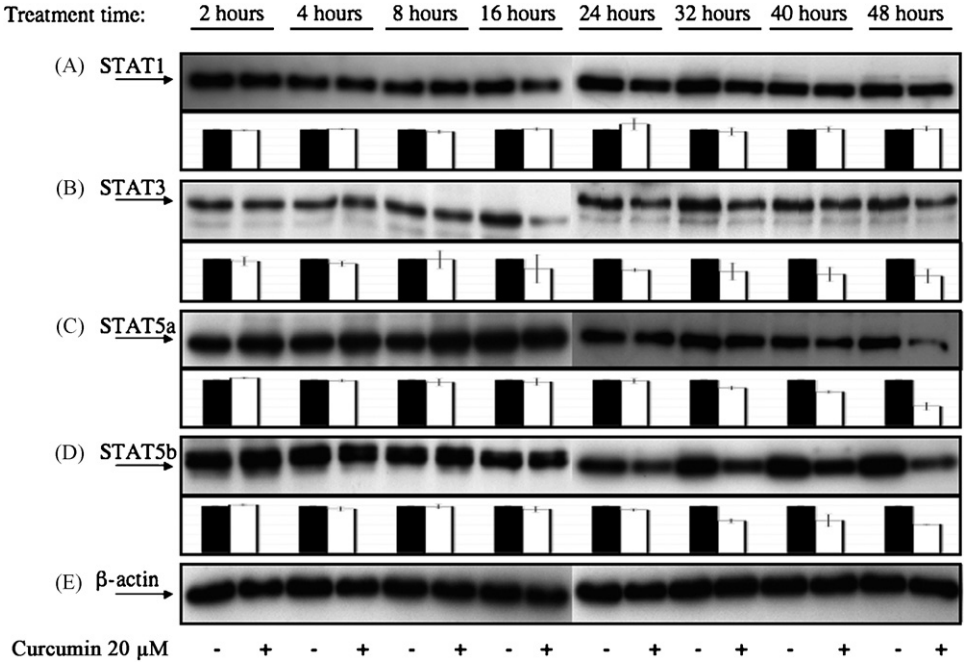

Fig. 1 – Effect of various times of curcumin treatment on nuclear STAT1, -3, -5a and -5b. Western blot analysis of the nuclear factors extracted from K562 cells after 2–48 h of 20 μM curcumin treatment. (A) Non-cross-reactive detection of the C-terminal part of STAT1; (B) non-cross-reactive detection of the central part of STAT3; (C) non-cross-reactive detection of the C-terminal part of STAT5a; (D) non-cross-reactive detection of the C-terminal part of STAT5b; (E) β-actin was used as loading control. Results are the mean of three independent experiments.

analyses were performed on nuclear extracts obtained after 48 h of treatment with increasing concentrations of curcumin (Fig. 2). Again, no variation in STAT1 expression could be observed after a 1, 5, 10 or 20 μM curcumin treatment. STAT3 expression remains constant at low curcumin concentrations as a significant decrease can only be observed with 10 or 20 μM curcumin treatments. The decrease of both STAT5a and STAT5b expression is also observed with the highest curcumin concentration. Levels of β-actin expression remained unchanged.

3.2. *Curcumin does not affect constitutive phosphorylation of STAT3 and -5*

Previous reports show that K562 cells constitutively express phosphorylated STAT proteins [22,23]. Western blots obtained with phosphospecific antibodies targeting STAT1, STAT3 and STAT5 confirm the presence of activated STAT3 and -5 proteins in the K562 cell line (Fig. 3, lanes 1 and 2). A 20 μM curcumin treatment during 2 h did not induce significant variations in the levels of phosphorylated STAT3 or -5.

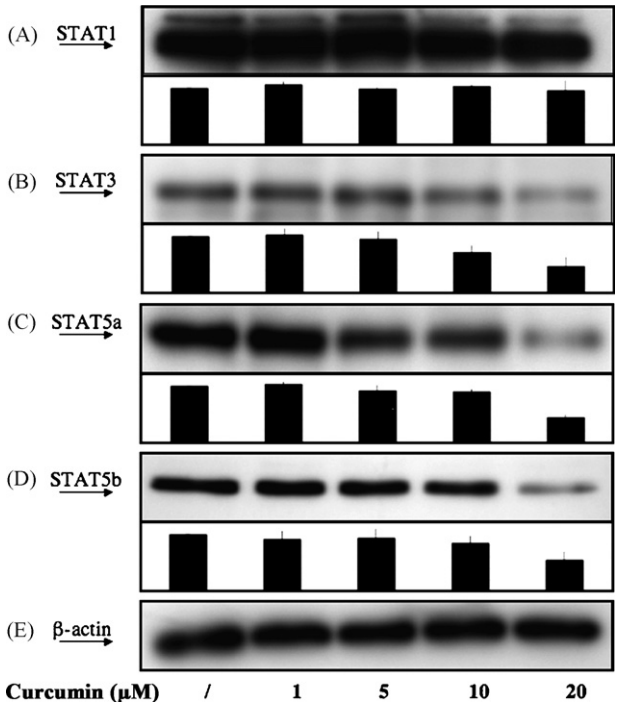

Fig. 2 – Effect of various concentrations of curcumin treatment on nuclear STAT1, -3, -5a and -5b. Western blot analysis of the nuclear factors extracted from K562 cells after 48 h of curcumin treatment. Treating concentrations used are: 0, 1, 5, 10 and 20 μM. (A) Non-cross-reactive detection of the C-terminal part of STAT1; (B) non-cross-reactive detection of the central part of STAT3; (C) non-cross-reactive detection of the C-terminal part of STAT5a; (D) non-cross-reactive detection of the C-terminal part of STAT5b; (E) β-actin was used as loading control. Results are the mean of three independent experiments.

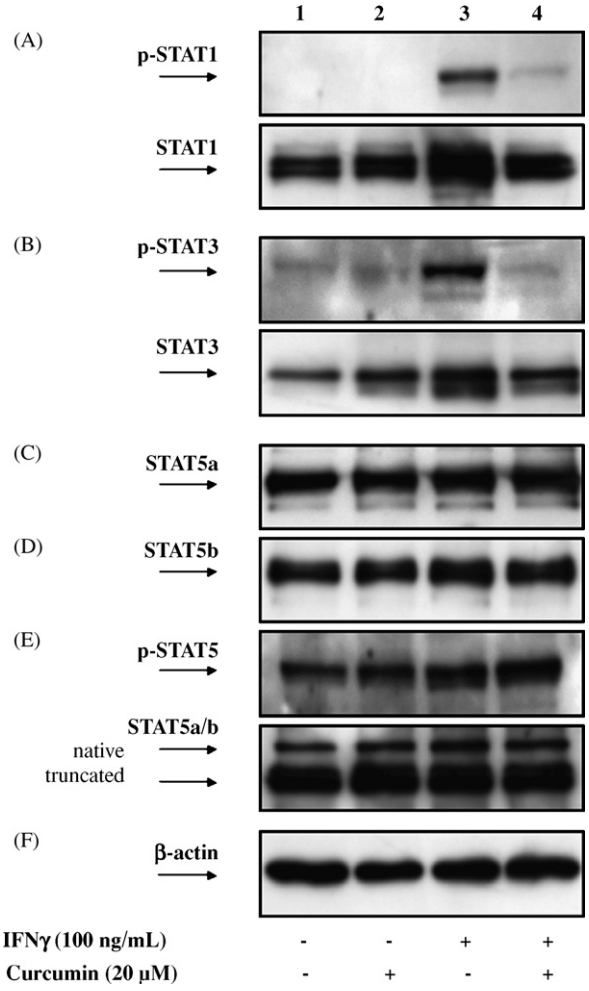

Fig. 3 – Effect of curcumin and IFN-γ treatments on nuclear STAT1, -3, -5a and -5b. Western blot analysis of the nuclear factors extracted from K562 cells treated for 6 h with or without IFN-γ (100 ng/mL) and pre-treated for 2 h with or without 20 μM curcumin. Antibodies used are specific for STAT1 (A), STAT3 (B), STAT5a (C), STAT5b (D) or the N-terminal part of STAT5 (E). β-Actin (F) was used as loading control.

3.3. *Curcumin generates truncated STAT5*

Previous studies showed the existence of naturally occurring carboxyl-truncated STAT5 proteins [24]. We thus used a STAT5 specific antibody targeting the conserved N-terminal region in order to study the possible formation of truncated STAT5a and b proteins (77 and 80 kDa) after curcumin treatment. Western blot analysis of the nuclear extracts obtained after 48 h of curcumin treatment show not only the presence of native STAT5a and b (92 and 94 kDa), but in addition a faster migrating species (≈80 kDa) corresponding to the truncated STAT5 (Fig. 4A). It can be noted that the latter isoform is already present in the nuclear extracts of untreated cells, showing the constitutive expression of truncated STAT5 proteins in the K562 cell line. The level of native STAT5 proteins decreases after 48 h of a 20 μM curcumin treatment, while the amount of cleaved STAT5 proteins increases after

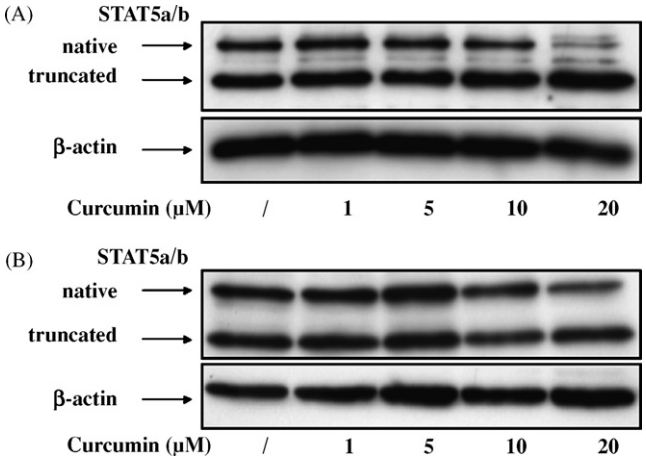

Fig. 4 – Effect of various concentrations of curcumin treatment on cytoplasmic and nuclear STAT1, -3, -5a and -5b. Western blot analysis of the cytoplasmic and nuclear factors extracted from K562 cells after 48 h of curcumin treatment. Treating concentrations used are: 0, 1, 5, 10 and 20 μM curcumin. Detection was performed by using an antibody specific of the N-terminal part of STAT5a and -5b. (A) Western blot of nuclear STAT5; (B) Western blot of cytoplasmic STAT5. β-Actin was used as loading control.

the same treatment, indicating that curcumin induces the conversion of native into truncated STAT proteins.

To assess subcellular localisation of truncated STAT5 isoforms in K562 cells, we performed western blot analyses of cytoplasmic proteins using the antibody targeting the N-terminal region of STAT5a and b. The results obtained show natural occurrence of truncated STAT5 isoforms in the cytoplasm (Fig. 4B). Furthermore, 20 μM curcumin treatment induces a decrease of native STAT5 and an increase of truncated STAT5 isoforms after 48 h.

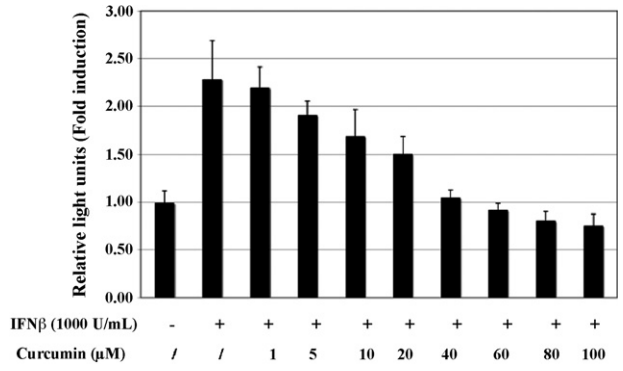

Fig. 5 – Effect of curcumin and IFN-β treatments on ISRE-luciferase reporter plasmid activity. K562 cells were transfected with an ISRE-luciferase reporter plasmid. Induction was performed with IFN-β (1000 U/mL) during 6 h. The effect of a 2 h curcumin pre-treatment was evaluated for a range of concentrations reaching from 1 to 100 μM.

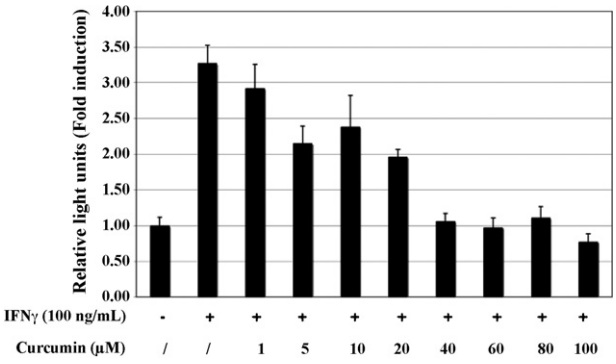

Fig. 6 – Effect of curcumin and IFN-γ treatments on ISRE-luciferase reporter plasmid activity. K562 cells were transfected with an ISRE-luciferase reporter plasmid. Induction was performed with IFN-γ (100 ng/mL) during 6 h. The effect of a 2 h curcumin pre-treatment was evaluated for a range of concentrations reaching from 1 to 100 μM.

3.4. *Curcumin interferes with STAT1 transactivation*

Type I or II IFN stimulation leads to the formation of STAT1–2 heterodimers, which can bind to the interferon regulatory factor 9 (IRF9) to form the interferon-stimulated gene factor 3 (ISGF3). The latter complex binds to interferon-stimulated response elements (ISREs) and activates their transcription [25,26]. Fig. 5 shows the IFN-β (1000 U/mL; 6 h) induced transcriptional activity in cells transfected with an ISRE reporter plasmid. When cells are pre-treated for 2 h with curcumin, a concentration-dependent decrease (35% for a 20 μM curcumin treatment) of the induced transcriptional activity of the ISRE plasmid is observed. The use of concentrations higher than 40 μM leads to a complete down-regulation of the IFN-β induction.

The same effect could be observed in K562 cells after IFN-γ (100 ng/mL; 6 h) treatment (Fig. 6). Indeed, IFN-γ induced ISRE transcriptional activity can be down-regulated by 2 h of curcumin pre-treatment at 20 μM. Again, this inhibition is strongly concentration dependent.

Nuclear extracts obtained from K562 cells treated with IFN-γ (100 ng/mL) for 6 h, with or without 2 h of 20 μM curcumin pre-treatment were analysed by Western blot. Fig. 3 (lanes 3 and 4) shows that a curcumin pre-treatment alone has no effect on the amount of neither of the STAT proteins analysed. IFN-γ treatment however induces an increase of the amount of nuclear STAT1 and STAT3 in K562 cells. This induction can be efficiently inhibited by a 20 μM curcumin pre-treatment. To the contrary, no variation in the amount of nuclear STAT5a or STAT5b could be detected, as well as these treatments did not influence the distribution of full-length or truncated STAT5 proteins in the nucleus of the K562 cell line.

3.5. *Curcumin reduces Jak2 expression and interferes with STAT target gene expression*

In order to complete our results, we investigated the effect of curcumin on genes that are regulated by STATs as well as the

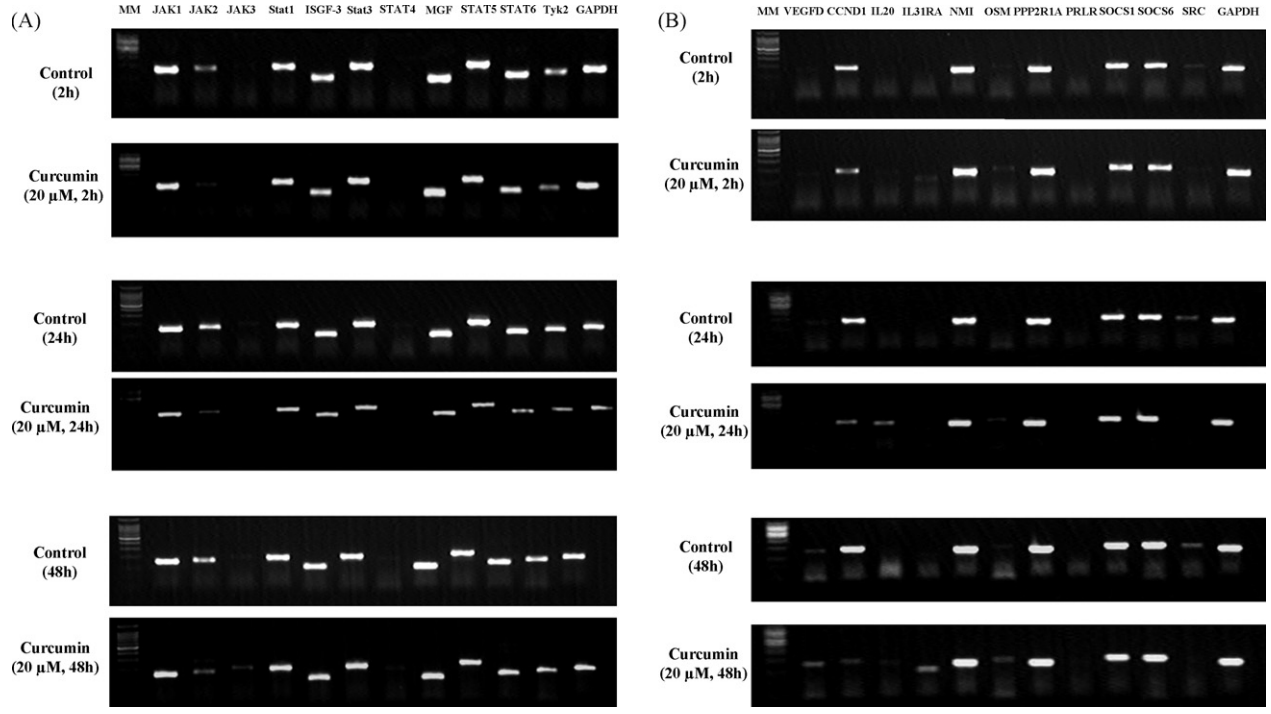

Fig. 7 – Effect of curcumin on mRNA expression. Total RNA, extracted from K562 cells with or without treatment with curcumin at 20 μM for indicated times, were submitted to reverse transcription (RT) and analysed by agarose gel electrophoresis (see Section 2 for details).

effect of curcumin on expression of Jak1, 2 and 3 as well as v-scr; interestingly, RT-PCR analysis of a panel of genes clearly shows reduction of the expression level of Jak2 after 2 h of treatment. v-src expression is also reduced as well as the cyclin D1 mRNA levels (Fig. 7A and B). Inhibition of these target genes did not change after 24 or 48 h of treatment. Finally, results show that mRNA levels encoding various Stat isoforms are not affected by curcumin treatment independently of the incubation time.

4. Discussion

Numerous reports described the effect of curcumin on human cancer and leukaemia cells. It is well established now that diferuloylmethane or curcumin is a strong inducer of apoptotic mechanisms underlining the potential of this natural chemopreventive agent as a non-toxic pharmacological agent. It was previously reported that curcumin induces apoptosis in human HL-60 promyelocytic leukaemia cells through mitochondrial pathway involving caspase-8, BID cleavage, cytochrome *c* release, and caspase-3 activation. Results also suggest that Bcl-2 and Bcl-xl are critical negative regulators of curcumin-induced apoptosis [27]. In human mantle cell lymphoma, curcumin inhibits the constitutive NF-κB and IKK leading to suppression of expression of NF-κB-regulated gene products that results in the suppression of proliferation, cell cycle arrest and induction of apoptosis. Finally, curcumin induces also apoptosis in acute T cell leukaemia via PI3′-kinase/AKT pathway [28], in follicular lymphoma cells lines [29] as well as in Jurkat T cell leukaemia.

In K562 chronic myelogenous leukaemia cells inhibition of glutathione S-transferase P1-1 expression [30] leads to induction of apoptosis. Nevertheless induction of caspase-8 and -9 cleavage is delayed and at concentrations used in this report, apoptotic mechanisms are not activated. Resistance of K562 cells towards apoptosis is largely due to the fact that this chronic leukaemia cell line has other oncogenic events besides Bcr-Abl including a lack of p53. Therefore, effects observed on K562 cells on STAT signaling mechanisms were not due to nonspecific toxic effects of curcumin.

Moreover, it seems that the STAT transcription factor appears to be an additional target for curcumin as Rajasingh et al. [31] recently described the antitumor effect of curcumin in human T cell leukaemia to be due to a dose-dependent decrease in JAK and STAT phosphorylation resulting in the induction of growth-arrest and apoptosis in T cell leukaemia. In primary effusion leukaemia, curcumin inhibits cell proliferation and induces apoptosis in a dose dependent manner. According to Uddin et al. [20], this observation appears to result from suppression of the constitutively active STAT3 through inhibition of Janus kinase 1.

In opposition to these studies, which target the phosphorylated form of the STAT proteins, we investigated the effect of curcumin on nuclear STAT1, -3 and -5. While no effect of curcumin on the nuclear amount of STAT1 could be seen, we were able to show for the first time a curcumin-induced decrease of the nuclear amount of STAT3, -5a and -5b. It is however noteworthy to mention that the treatment times needed to observe these variations are quite long, and that such prolonged treatment times do not influence the phosphorylation status of these proteins in K562 cells.

The absence of dephosphorylation after curcumin treatment can have several origins, as on one hand the amount of phosphorylated STATs detected in the K562 cell line is rather low, and on the other hand the dephosphorylation process appears at much shorter time scales.

Previous studies in the literature show that truncated STAT5a and -5b can be generated by proteolytic cleavage or by alternative splicing [32]. However, the loss of their C-terminal transactivation domain does not prevent STAT5 DNA-binding ability, and these truncated proteins can act as dominant negative competitors of STAT5a and -5b [24,32–36]. Such cleaved STAT5 isoforms, termed STAT5a p77 and STAT5b p80, were also observed under native cell conditions [24,33]. The formation of these truncated STAT5 isoforms can both stem from alternative splicing or be caused by a protease, which cleaves the full-length STAT5 isoforms and removes the C-terminal part of the proteins [37]. The proteolytic activity has been assigned to the nucleus in the case of early haematopoietic progenitors [37,38], while studies on acute myeloid leukaemia blasts showed proteolytic activity in the nucleus and in the cytoplasm [39]. This difference was explained by the existence of different proteases depending on the cell line type studied. Our results confirm that these STAT5 proteins are truncated at the C-terminal part, as they can only be detected with the antibodies targeting the N-terminal part of the protein, but not with the C-terminal specific ones.

Furthermore, our results obtained with the K562 cell line show that the truncated STAT5 isoforms are not sequestrated in the nucleus, but are also expressed in the cytoplasm. Interestingly, curcumin treatment induces a decrease of full-length STAT5 and an increase of truncated ones. Finally, the effect of curcumin treatment on STAT5 is not only observable in the nucleus as variations of its amounts can also be observed in the cytoplasm. When taking in account the fact that the truncated STAT5 proteins are known to behave as dominant negative regulators of full length STAT5, curcumin could be an interesting molecule for treatment in cancers over-expressing STAT5. Furthermore, it is interesting to observe that although long curcumin treatments decrease the amount of total STAT5, they do not influence the amount of phosphorylated STAT5.

As curcumin had no regulatory effect on the amount of unphosphorylated nuclear STAT1, we investigated its influence on the induced transcriptional activity of the STAT1–2 heterodimer. The results obtained show that even if curcumin has no effect on constitutive STAT1, it is able to inhibit efficiently the IFN-β and IFN-γ induced STAT1–2 transcription activity as well as the induced phosphorylation of STAT1 and STAT3. These results are in agreement with previous ones obtained in brain microglia, showing that curcumin treatment inhibits IFN-γ induced STAT1 and -3 phosphorylation [17].

CML cells are characterized by a close interaction between Bcr-Abl and Jak2, which are activating a cell signaling cascade also involving PI-3K as well as AKT and Gsk-3b. Eventually activation of this cascade leads to overexpression of c-Myc. Accordingly, Samanta et al. [Cancer Res] described recently Jak2 as a critical target in CML treatment. Whereas those authors use a chemical inhibitor AG490 to inhibit Jak2 gene expression, our RT-PCR experiments show that curcumin is able to completely inhibit the expression of Jak2 at the level of transcription. Future investigation will further detail the use of curcumin as an inhibitor of Jak2 expression.

Taken together, our results show that curcumin is a potent inhibitor of both constitutive and induced STAT proteins as well as Jak2, and that this chemopreventive agent could play an interesting role in cancer treatments with or without classic anti-cancer drugs.

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