

Cell swelling activates STAT1 and STAT3 proteins in cultured rat hepatocytes

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Abstract In this paper, we demonstrated that in cultured rat hepatocytes cell swelling induced the activation of STAT1 and STAT3 proteins without any effect on STAT4, STAT5 and STAT6 proteins. Cell swelling induced an activation of STAT proteins through an increase in the phosphorylation of the tyrosine residue also phosphorylated by interleukin-6, but without any activation of JAK kinases. The signaling pathway by which cell swelling activated STAT1 and STAT3 is discussed.

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Key words: STAT protein; Hypoosmolarity; α 2-Macroglobulin; Interleukin-6; Swelling; Hepatocyte

1. Introduction

Change in cell volume is now regarded as a novel mechanism modulating gene expression (for review see [1]). Thus, in the liver, cell swelling has been reported to increase the level of the mRNAs for cytoskeleton proteins such as β -actin [2] and tubulin [3], for the enzymes argininosuccinate synthetase [4] and ornithine decarboxylase [5], for the excreted protein α 2-macroglobulin (α 2M) [6] and the transcription factor c-jun [7]. Moreover, cell swelling was reported to decrease the mRNA level for the enzymes phosphoenolpyruvate carboxykinase and tyrosine aminotransferase [8] and for excreted proteins such as the α 2-HS-glycoprotein [9]. The changes in the mRNA level have been shown to be associated with corresponding changes in the transcription of genes such as those of β -actin [10] and α 2M [6] in adult rat hepatocytes and of phosphoenolpyruvate carboxykinase in hepatoma cells [11]. However, the transcription factor(s) involved in these effects have not yet been identified.

The aim of this work was to test the possibility that the STAT (signal transducer and activator of transcription) transcription factors might be involved in the effect of cell swelling in cultured rat hepatocytes. The obtained results demonstrate that cell swelling activates STAT1 and STAT3 but not STAT4, STAT5 and STAT6 proteins in primary cultured rat hepatocytes. Since STAT1 and STAT3 are the known factors of the inducing effect of interleukin-6 (IL-6) on the α 2M gene [12,13], these results might therefore explain the transcriptional effect of cell swelling on this gene [6]. The

mechanism by which cell swelling activated STAT1 and STAT3 proteins is discussed.

2. Materials and methods

2.1. Materials

Dexamethasone, raffinose and 2-aminoisobutyric acid were purchased from Sigma and recombinant human interleukin 6 (rh-IL-6) from Boehringer Mannheim. Interferon- α (IFN α) was kindly provided by S. Pellegrini [14]. Fetal calf serum was from Dutscher (Brumath, France). sgp80 purified from baculovirus was kindly provided by J. Brakenhoff (CLB Amsterdam). Hybond-N membranes, multi-prime DNA labelling, [α -³²P]dCTP (specific radioactivity, 3000 Ci/mmol), Hyperfilm-MP and chemiluminescence reagent were from Amersham. The antibodies used were the following: anti-JAK1 C-terminal fusion protein [15] (gift from A. Ziemiecki); anti-JAK2 C-terminal peptide (Upstate Biotechnology Inc.); anti-TYK2 fusion protein [14]; anti-STAT1 N-terminal domain (anti-ISGF3 p91/p84 monoclonal antibody, Transduction Laboratories, UK); anti-STAT3 C-terminal peptide (30C, gift from D. Levy, New York University Medical Center) [16]; anti-STAT4 C-terminal peptide (C-20, Santa Cruz Biotechnology Inc.); anti-full length human STAT6 (gift from Steve McKnight, Tularik, Inc.), anti-phosphotyrosine monoclonal 4 G10 (gift from B. Drucker, Portland) and anti-STAT3 (Ptyr 705) (Biolabs).

2.2. Cell culture

Isolated hepatocytes from 24-h starved male adult Wistar rats (200–220 g) were prepared as described [17]. Hepatocytes were suspended in the culture medium (199 medium/MEM, 25%:75%) containing 10% fetal calf serum. The cells were seeded in 100 mm diameter plastic dishes pre-coated with 400 μ g rat tail (type I) collagen, and cultured at 37°C under 5% CO₂ in air. After an attachment period of 4 h, the medium was replaced by fresh culture medium deprived of glutamine containing 5% fetal calf serum and 10⁻⁷ M dexamethasone (control cells). Hepatocytes were next cultured in isoosmotic medium with 100 U/ml IL-6, the concentration required for the maximal inducing effect of IL-6 on the α 2M mRNA level, or with 10 mM AIB (2-aminoisobutyric acid) for various periods of time at 37°C. Hypoosmotic and hyperosmotic media were obtained respectively by decreasing (–50 mM) and increasing (+20 mM) the NaCl concentration of the medium. After the indicated times of treatment, the cells were pelleted and washed in cold phosphate-buffered saline (PBS).

Cell lines HepG2 [9], HT1080 and 3B [18] were cultured in DMEM containing 10% heat-inactivated fetal calf serum, streptomycin (50 mg/l) and penicillin (50 000 U/l).

2.3. Extraction and analysis of cellular RNA

Isolation of total RNA was performed by a guanidinium-thiocyanate procedure [19]. RNA was separated on 1.5% agarose/formaldehyde gels and transferred to nylon membranes for Northern hybridization. The membranes were hybridized with a random-oligonucleotide-primed ³²P-labeled DNA fragment as described [20]. The probes used were a fragment of rat α 2M cDNA provided by Dr. G. Fey [21] and a fragment of 18S rRNA provided by Dr. L. Hendricks [22]. Relative densities of the hybridization signals were quantified by scanning the films with a Biocom densitometer. To correct for differences in RNA loading, all the results were expressed as the ratio of the

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scanned values for $\alpha 2\text{M}$ mRNA vs. those for 18S rRNA (relative level).

2.4. Electrophoretic mobility shift assays (EMSA)

Nuclear extracts were prepared by a brief lysis of the cells in a mild buffer (20 mM HEPES pH 7.9, 10 mM KCl, 1 mM EDTA, 0.2% glycerol, 1 mM DTT, 1 mM PMSF, 1 mM Na_2VO_4) followed by extraction of the pelleted nuclei with a hypertonic buffer containing 350 mM NaCl, 20% glycerol, 20 mM HEPES pH 7.9, 10 mM KCl, 1 mM EDTA, 1 mM PMSF, 1 mM Na_2VO_4 . The extracts were centrifuged at $20\,000\times g$ for 5 min and supernatants were immediately frozen in liquid nitrogen and stored at -80°C before use. EMSAs were performed as described [23]. Oligonucleotide sequences known to bind STAT factors (from the c-fos promoter (5'-GTCGACAGTTCCCGTCAATC-3'), $\alpha 2$ -macroglobulin-APRE (5'-GATCCTCTGGGAATTCCTA-3'), IRF-1GAS (5'-GATCCATTTCCCGCAATGA-3') and β -casein promoter (5'-AGATTTCTAGGAATCAAATC-3')) were end-labeled with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. Extracts (from 3×10^5 cells/point) were incubated for 30 min at 4°C with the indicated ^{32}P -labeled oligonucleotide probe (about 30 000–40 000 cpm). Complexes were separated on 4% non-denaturing polyacrylamide gels in $0.25\times\text{TBE}$ and detected by autoradiography. For competition assays, unlabeled oligonucleotides were added at $50\times$ molar excess with the probe. Supershift assays were performed by incubating nuclear extracts with an excess of the indicated antibodies and the probe.

2.5. Western blot

Nuclear extracts prepared as above were subjected to SDS-PAGE (7.5%) under reducing conditions and transferred to nitrocellulose membrane for immunoblotting. Filters were blocked in TBS (10 mM Tris-HCl (pH 7.5), 137 mM NaCl) containing 0.2% Tween and 5% low fat dry milk for 2 h on a rocking platform. Blots were incubated with anti-tyr705-phosphorylated STAT3 Ab (1:600) for 18 h at 4°C . The bound antibodies were visualized by using ECL chemiluminescence according to the manufacturer's instructions.

2.6. Immunoprecipitations and immunoblotting

Cells (2×10^6 cells/point) were washed in PBS and solubilized in a RIPA buffer (50 mM Tris-HCl (pH 8.0), 200 mM NaCl, 0.05% SDS, 1% NP40, 0.5% NaDOC, 1 mM Na_2VO_4 , 1 mM PMSF, 10 $\mu\text{g}/\text{ml}$ pepstatin A, 10 $\mu\text{g}/\text{ml}$ leupeptin, 10 $\mu\text{g}/\text{ml}$ aprotinin) 30 min at 4°C . Insoluble material was pelleted at $20\,000\times g$ for 20 min and supernatants were immediately frozen in liquid nitrogen and stored at -80°C before use (total extracts). In some experiments, cells were treated with 100 μM Na_2VO_4 plus 10 μM H_2O_2 for 2 min before protein extraction. For immunoprecipitations, cell lysates were pre-incubated with protein G-Sepharose beads for 30 min at 4°C and pre-cleared supernatants were incubated with the indicated antibodies (1 μl of anti-JAK1, 2 μl of anti-JAK2 and 2 μl of anti-TYK2) for 18 h at 4°C . Immune complexes were collected by incubation with protein G-Sepharose beads and eluted by boiling in SDS sample buffer (0.0625 M Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, 5% β -mercaptoethanol). Immunoprecipitates were subjected to SDS-PAGE (8%) under reducing conditions and transferred to nitrocellulose membrane for immunoblotting. Nitrocellulose filters were blocked in TBS containing 0.1% Tween and 1% gelatin for 2 h on a rocking platform. Blots were incubated with antiphosphotyrosine 4 G10 mAb (1:10 000) for 18 h

at 4°C . Immune complexes were detected by ECL chemiluminescence according to the manufacturer's instructions.

2.7. Expression of the results

The results are expressed as means $\pm$ S.E.M. of the indicated number (n) of different cell preparations. Statistical significance of differences was calculated by Student's t -test for paired data.

3. Results and discussion

3.1. Hypoosmolarity induces STAT1 and STAT3 protein activation

In order to study whether hypoosmolarity resulted in activation of STAT proteins, we first performed EMSAs with the m67SIE probe known to bind STAT factors. Nuclear extracts prepared from hepatocytes cultured for 60 min in isoosmotic medium or in hypoosmotic medium, which induces cell swelling, were incubated with the m67SIE probe. Although a DNA binding activity was present in nuclear extracts from untreated cells, hypoosmolarity still induced an increased DNA binding activity in treated cells corresponding to two complexes migrating as STAT1/3 and STAT3/3 complexes (Fig. 1A); this was observed in six independent experiments. The specificity of DNA binding was confirmed by competition assays with an excess of unlabeled oligonucleotides (data not shown). The identity of the STATs activated by hypoosmolarity was assessed by supershift assays using anti-STAT1 and anti-STAT3 antibodies. The obtained results showed that antibody addition induced a disappearance of STAT3 protein as illustrated in Fig. 2. The same results were obtained using anti-STAT1 antibody (data not shown). Thus, hypoosmolarity activated STAT1 and STAT3 proteins with the formation of STAT3/3 and STAT1/3 dimers, preferentially. These results were confirmed using another probe, namely the APRE probe which is the acute phase responsive element of the $\alpha 2\text{M}$ gene promoter and known to bind STAT1 and STAT3 proteins (Fig. 1B).

Since no information was available regarding the nuclear factors involved in the effect of hypoosmolarity on any gene expression, we also studied the effect of hypoosmolarity on the other STATs which are expressed in rat liver, namely STAT4, STAT5 and STAT6. Using the nuclear extracts prepared in the experiments described above, we performed EMSAs with oligonucleotides known to bind these STATs. EMSAs performed with the β -casein probe that preferentially binds STAT5 and to a weaker extent STAT6 showed no DNA binding activity at any time of hypoosmolarity treatment, as illustrated in Fig. 1C after 60 min of treatment.

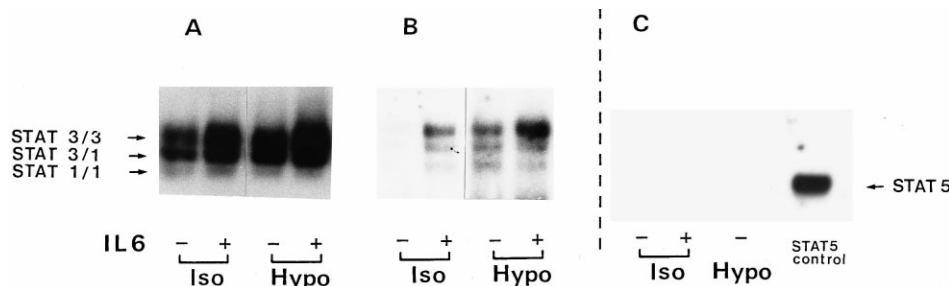


Fig. 1. Influence of hypoosmolarity on STAT1, STAT3 and STAT5 proteins. Hepatocytes were cultured for 60 min in isoosmotic medium (Iso) or in hypoosmotic medium (Hypo) in the absence (–) or in the presence (+) of 100 U/ml IL-6 (IL-6). Nuclear extracts were analyzed by EMSA using the m67SIE probe (A), the APRE probe (B) and the β -casein probe (C). UT7 mpl cells cultured in the presence of GM-CSF and treated with TPO for 30 min were used as STAT5-activated control [23].

EMSA were also performed with the IRF-1GAS oligonucleotide as a probe. Although activation of STAT4 and STAT6 was observed in untreated cells, we were unable to detect any significant change under the influence of hypoosmolarity (data not shown). This demonstrated that hypoosmolarity induced the activation of STAT1 and STAT3 but did not activate STAT4, STAT5 and STAT6 proteins. Since hypoosmolarity has been shown to induce an increase in the transcription of the $\alpha 2M$ gene [6], this suggested that the activation of both STAT3 and STAT1 might contribute to this inducing effect.

In order to specify the modalities of action of hypoosmolarity, we compared the kinetic of STAT1 and STAT3 activation, and that of the $\alpha 2M$ mRNA expression following hypoosmotic or IL-6 treatments. Hepatocytes were cultured for various periods of time in isoosmotic medium with or without IL-6, or in hypoosmotic medium. As shown in Fig. 3A, hypoosmolarity induced a time course of STAT1 and STAT3 activation similar to that induced by IL-6, i.e. a significant increase at 30 min of culture with a maximal increase at 60 min (see also Figs. 1A and 2). Similarly, the activation of the STATs was associated with expression of the $\alpha 2M$ gene which was detectable after 3 h of culture in both isoosmotic medium with IL-6 and hypoosmotic medium (Fig. 3B). This therefore suggested that hypoosmolarity and IL-6 might induce the expression of the $\alpha 2M$ gene through a similar activation of STAT1 and STAT3 proteins.

Concerning the influence of hypoosmolarity on $\alpha 2M$ gene expression, we also reported that the effect of hypoosmolarity was additive to that of IL-6 [6]. We therefore started experiments in order to specify if this was also true for the activation of STAT1 and STAT3 proteins. Hepatocytes were cultured for 60 min in isoosmotic medium or in hypoosmotic medium with or without 100 U/ml IL-6. As illustrated in Fig. 1A, EMSAs using the m67 SIE probe showed that hypoosmolarity reinforced the effect of IL-6 on the activation of STAT1 and STAT3 proteins. This was confirmed using the APRE probe (Fig. 1B). Therefore, these results demonstrated that hypoosmolarity reinforced the effect of IL-6 on STAT1 and STAT3 proteins. This suggested that the additivity of the effects of IL-6 and hypoosmolarity on $\alpha 2M$ gene expression might be a consequence of the additivity of their effect on STAT1 and STAT3 protein activation.

Since the cell swelling induced by hypoosmolarity was shown to be responsible for its effect on the regulation of $\alpha 2M$ gene expression [6], we started experiments in order to demonstrate that cell swelling per se was truly responsible for STAT1 and STAT3 activation.

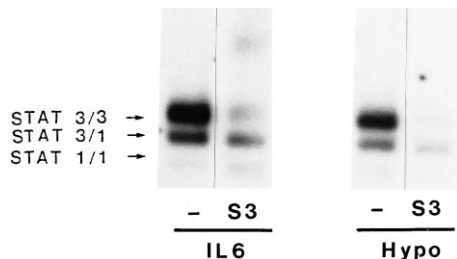


Fig. 2. Supershift analysis by anti-STAT3 antibody. Hepatocytes were cultured for 60 min in hypoosmotic medium (Hypo) or in isoosmotic medium with 100 U/ml IL-6 (IL-6). Nuclear extracts were analyzed by EMSA using the m67SIE probe in the absence (–) or in the presence of anti-STAT3 (S3) antibody.

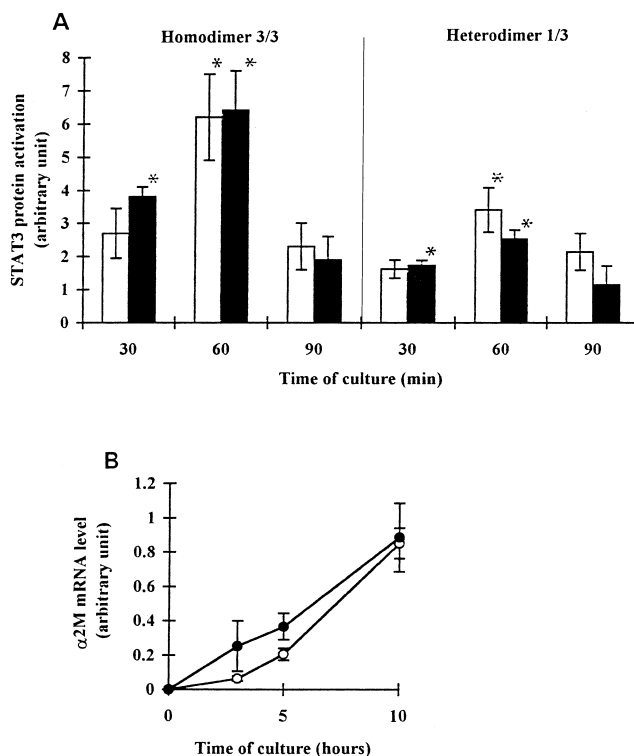


Fig. 3. Comparison of the effects of hypoosmolarity and IL-6 on STAT1 and STAT3 activation, and on $\alpha 2M$ gene expression. A: Nuclear extracts from hepatocytes cultured in isoosmotic medium with 100 U/ml IL-6 (□) or in hypoosmotic medium (■) for 30, 60 and 90 min were analyzed by EMSA using the m67SIE probe. STAT3/3 homodimer and STAT1/3 heterodimer signals were quantitated by densitometry and expressed relative to the signals measured for the control value in each experiment. The results are means $\pm$ S.E.M. for three independent experiments. *Significantly different ($P < 0.05$) from the corresponding control values. B: Hepatocytes were cultured in isoosmotic medium with 100 U/ml IL-6 (○) or in hypoosmotic medium (●) for 3, 5 and 10 h. Total RNA was extracted and 25 μ g aliquots were analyzed by Northern blot. Blots were probed successively with the $\alpha 2M$ and the 18S cDNAs. The results are means $\pm$ S.E.M. for five independent experiments.

3.2. Cell swelling is responsible for STAT1 and STAT3 activation

The hypoosmotic medium used in the above experiments was obtained by decreasing the NaCl concentration. Thus, we could not exclude that variation in NaCl concentration per se induced the observed activation of STAT1 and STAT3. We therefore restored the cell volume to normal conditions in the presence of the same low NaCl concentration by adding 100 mM raffinose. As shown in Fig. 4A, addition of raffinose to low NaCl-hypoosmotic medium inhibited the activating effect of hypoosmolarity on STAT1 and STAT3 (1, iso; 2.0, hypo; 1.2, hypo+raffinose; $n=2$, for the STAT3/3 dimer). This demonstrated that the activating effect of hypoosmolarity on STATs cannot be a consequence of the change in NaCl concentration. In order to confirm that cell swelling was the true mechanism involved in the effect of hypoosmolarity on STAT activation, we also tested the influence of AIB. AIB is a non-metabolizable amino acid analog which enters the cell using a sodium-dependent transport that triggers cell swelling. Hepatocytes were therefore cultured in isoosmotic medium with or without 10 mM AIB for 60 min. Fig. 4B shows that AIB induced an activation of STAT1 and

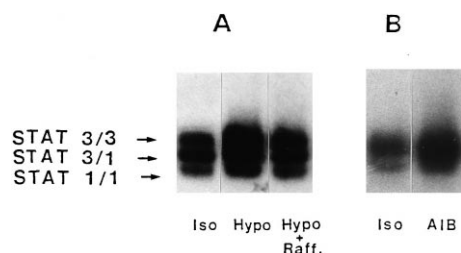


Fig. 4. Influence of cell swelling on STAT1 and STAT3 proteins. Hepatocytes were cultured for 30 min in isoosmotic medium (Iso), or in hypoosmotic medium with (Hypo+Raff) or without (Hypo) 100 mM raffinose (A), and for 60 min in isoosmotic medium with (AIB) or without (Iso) 10 mM AIB (B). Nuclear extracts were analyzed by EMSA using the m67SIE probe.

STAT3; this was observed in two independent experiments. Moreover, we recently reported that AIB induced the expression of the $\alpha 2M$ gene [24]. Taken together, all these results demonstrate that the activation of STAT1 and STAT3 proteins correlates with the induction of the expression of the $\alpha 2M$ gene by cell swelling inducers.

Since IL-6 induces the activation of STAT proteins through their phosphorylation on a tyrosine residue [25], we started experiments in order to determine if the effect of cell swelling might involve such a mechanism.

3.3. Cell swelling acts through an increase in the phosphorylation of the tyrosine residue involved in the effect of IL-6 on STAT3 protein

IL-6 induces the activation of STAT1 and STAT3 proteins by phosphorylation on a critical tyrosine residue: tyrosine 701 for STAT1 and tyrosine 705 for STAT3. We therefore studied the effect of cell swelling on the specific tyrosine involved and focused on the phosphorylation of STAT3. Hepatocytes were cultured for 60 min in isoosmotic medium with or without 100 U/ml IL-6 or in hypoosmotic medium. Nuclear extracts were prepared and analyzed by Western blot analysis using anti-STAT3 antibody specific for the STAT3 protein phosphorylated on tyrosine 705. As shown in Fig. 5, although phosphorylation on tyrosine 705 was detected in untreated cells, hypoosmolarity induced an increase in the phosphorylation on this specific tyrosine. Moreover, this figure also shows that the effect of hypoosmolarity was additive to that of IL-6 on tyrosine 705. These results demonstrated that cell swelling induced an activation of the STAT3 protein in the absence or in the presence of IL-6 through an increase in the phosphorylation of the tyrosine residue specifically involved in the effect of IL-6.

IL-6 activates kinases belonging to the JAK family, namely JAK1, JAK2 and Tyk2 [26,27], leading to the tyrosine phosphorylation of STAT1 and STAT3 proteins. We therefore tested the possibility that these kinases may be involved in the effect of cell swelling.

3.4. JAK kinases are not involved in the effect of cell swelling

In order to assess whether cell swelling might increase tyrosine phosphorylation of STAT proteins through an activation of JAKs, we started experiments on cultured rat hepatocytes. Since JAK kinases are activated through tyrosine phosphorylation, we studied the effect of hypoosmolarity on their phosphorylation on tyrosine residues. Unfortunately,

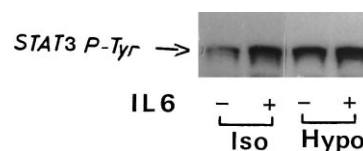


Fig. 5. Influence of cell swelling on the phosphorylation of the STAT3 protein on tyrosine 705. Hepatocytes were cultured for 60 min in isoosmotic medium (Iso) or in hypoosmotic medium (Hypo) in the absence (–) or in the presence (+) of 100 U/ml IL-6 (IL-6). Nuclear extracts were subjected to SDS-PAGE 7.5%. Blots were probed with anti-tyr705-phosphorylated STAT3 antibody.

even in the presence of high concentrations of IL-6 (10 000 U/ml) and in the presence of its soluble receptor (sgp80), we did not detect any significant tyrosine phosphorylation on any JAK tested (JAK1, JAK2 and Tyk2) in hepatocytes in primary culture at any time tested (i.e. 5, 10 and 15 min). We were therefore unable to specify if JAKs are involved in the effect of cell swelling. In order to assess whether cell swelling might activate JAK kinases, we started experiments using other cell types. We first studied the influence of hypoosmolarity in human hepatoma cells (HepG2) known to be responsive to IL-6. HepG2 were treated for 5 or 15 min in isoosmotic medium in the presence of 1000 U/ml IL-6 or in hypoosmotic medium, and the tyrosine phosphorylation of Tyk2 was studied. As shown in Fig. 6A, treatment of HepG2 cells for 15 min in the presence of IL-6 induced an increase in the phosphorylation of Tyk2, as expected. However, no increase was detected under the influence of hypoosmolarity. Since Tyk2 was slightly phosphorylated in the presence of IL-6 using HepG2, we next studied the effect of hypoosmolarity in human fibrosarcoma cells overexpressing Tyk2 (3B cells). As shown in Fig. 6B, the treatment of 3B cells for 15 min with 1000 U/ml IFN α induced a strong phosphorylation of Tyk2. However, we were again unable to detect any activation of Tyk2 under the influence of hypoosmolarity, demonstrating that cell swelling did not induce any activation of the kinase. Then, we studied the influence of hypoosmolarity on the phosphorylation of JAK1, the main kinase involved in the effect of cytokines on STAT phosphorylation [28], in HT1080 cells. Although 1000 U/ml IFN α used to activate JAK1 induced an increase in the phosphorylation of the kinase, we were unable to detect any increase in

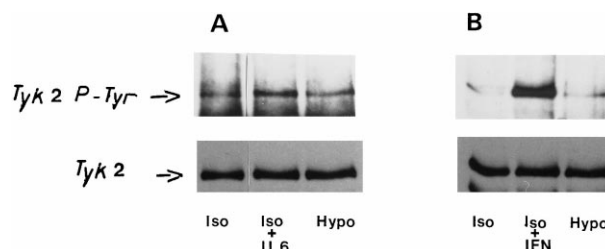


Fig. 6. Influence of hypoosmolarity on the phosphorylation of Tyk2 on tyrosine residues. A: HepG2 cells were cultured for 15 min in isoosmotic medium with (Iso+IL-6) or without (Iso) 1000 U/ml IL-6, or in hypoosmotic medium (Hypo). B: 3B cells were cultured for 15 min in isoosmotic medium with (Iso+IFN) or without (Iso) 1000 U/ml IFN α , or in hypoosmotic medium (Hypo). Total extracts were immunoprecipitated with anti-Tyk2 antibody (2 μ l) and subjected to 8% SDS-PAGE. Tyrosine phosphorylation was assessed by immunoblotting with antiphosphotyrosine antibody 4G10. Blots were re-probed with anti-Tyk2 antibody. The position of the Tyk2 protein is indicated.

the tyrosine phosphorylation under the influence of hypo-osmolarity (data not shown).

Taken together, all these results suggest that the activation of JAK kinases is not involved in the phosphorylation of STAT proteins under the influence of cell swelling. This was also reinforced by the fact that, in contrast, cell shrinkage was recently reported to activate JAK1, JAK2 and Tyk2 in different cell lines including HepG2 [29]. This therefore suggested that cell swelling may act through activation of other tyrosine kinases such as Src [30] and (or) inhibition of a tyrosine protein phosphatase.

4. Conclusion

The data reported here clearly demonstrate that cell swelling per se induces an activation of the proteins STAT1 and STAT3 through their phosphorylation on the tyrosine residue which is also phosphorylated by IL-6, at least for STAT3. Thus, the inducing effect of cell swelling on the expression of the $\alpha 2M$ gene may be explained by the activation of these transcription factors. Concerning the signaling pathway involved, our results also demonstrate that JAK kinases are not involved in the effect of cell swelling suggesting that cell swelling may act through activation of other tyrosine kinases and (or) inhibition of protein tyrosine phosphatases. Interestingly, Gatsios et al. recently reported that, in contrast, cell shrinkage also induced the activation of STAT1 and STAT3 but through an activation of JAKs in different cell lines including HepG2 [29]; this was only observed following an extremely strong increase in osmolarity (+400–600 mM sorbitol corresponding to about 200–300 mM NaCl added to the iso-osmotic medium). Taken together, these results therefore suggest that osmotic stress induced by either a decrease or a strong increase in osmolarity might induce STAT1 and STAT3 activation. Similar conclusions were reported for another nuclear transcriptional factor in HeLa cells, namely HSF1 [31]. Thus, cell swelling and cell shrinkage may activate the same transcription factors but by different signaling pathways. However, the signaling pathway involved in the effect of cell swelling remains to be identified.

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