SMAD proteins are involved in apoptosis induction in ventricular cardiomyocytes

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Abstract

Objective: The transcription factor AP-1 is a mediator of hypertrophic growth and apoptosis in cardiomyocytes. This puts AP-1 in the center of two important processes found in the failing heart and implies that variations (i) in the AP-1 composition itself or (ii) in additional, interacting transcription factors are responsible for the diverse actions of AP-1. To test this hypothesis, we performed studies on isolated ventricular cardiomyocytes of rat under hypertrophy- or apoptosis-inducing conditions.

Methods and results: The NO donor SNAP (100 μM), which is a pro-apoptotic stimulus in cardiomyocytes, activated AP-1 within 2 h. c-Jun, JunB and FosB are identified as the main components of this AP-1 complex. This complex formation is identical to the composition of AP-1 found under hypertrophic growth stimulation by phenylephrine (PE, 10 μM). Analysis of other transcription factors able to interact with AP-1 revealed activation of SMAD activity only during stimulation with SNAP to 131 ± 9.6% (p < 0.05 vs. control, n = 9). The SMAD complex is formed from SMAD4 and 3. Intracellular scavenging of SMAD proteins by transformation of cardiomyocytes with SMAD decoy oligonucleotides or inhibition of SMAD4 synthesis using SMAD4 antisense oligonucleotides reduced the number of apoptotic cells under stimulation with SNAP from 13.3 ± 1.2% to control levels (8 ± 1%, p < 0.05, n = 6). TGFβ, which is a known stimulator of SMAD proteins, is also shown to stimulate apoptosis in cardiomyocytes. Again, simultaneous activation of AP-1 and SMAD is needed for this apoptosis induction.

Conclusions: In conclusion, AP-1/SMAD signaling has been identified as a common pathway in cardiomyocyte apoptosis. In contrast, SMAD proteins are dispensable for AP-1-mediated hypertrophic growth. This finding characterizes SMAD proteins as potential candidates for proteins that shift AP-1 signaling from hypertrophy to apoptosis.

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1. Introduction

During the development of heart failure, hypertrophic growth, which is followed by apoptosis, results in a very unfavorable remodeling of the ventricles. Little is known at present about the putative molecular switches that may lead cardiomyocytes from the route of hypertrophy to apoptosis.

Interestingly, the transcription factor AP-1 is a mediator of both processes, hypertrophy and apoptosis, in cardiomyocytes [1,2]. Differences in the composition of the AP-1 complex or in the interactions of AP-1 with additional transcription factors may be responsible for the induction of either hypertrophy or apoptosis via AP-1. Identification of such factors that can influence AP-1 activity may therefore help to characterize triggers responsible for the switch between cardiac hypertrophy and apoptosis.

Correlations between development of hypertrophy and induction of AP-1 have been demonstrated in several models of cardiac hypertrophy, e.g., in stroke-prone spontaneously hypertensive rats [3], in double transgenic renin-angiotensin mice [4], or in isoprenaline infused rats [5]. Functional...
involvement of AP-1 in the process of hypertrophic growth has been shown in isolated cardiomyocytes: in neonatal cardiomyocytes, \( \alpha \)-adrenergic stimulation was shown to promote growth and activate AP-1. This effect could be inhibited by overexpression of dominant negative mutants of c-Jun, which is a subunit of the AP-1 dimer [6]. In adult cardiomyocytes, we showed previously that \( \alpha \)-adrenergic stimulation induces growth and activates AP-1. We also demonstrated that these events are causally related since the growth response could be abolished by decoy oligonucleotides inhibiting AP-1 binding [2].

Concomitant activation of AP-1 and induction of apoptosis in the heart have been shown in several models of ischemia/reperfusion injury [7,8]. In isolated cardiomyocytes, AP-1 was found to be activated when the cells were driven into apoptosis, i.e., by oxygen radicals or nitric oxide. Overexpression of dominant negative c-Jun mutants inhibited induction of apoptosis by oxygen radicals in cardiac cells [9]. We showed recently direct evidence for a causal role of AP-1 in the induction of apoptosis during exposure to nitric oxide (NO) in ventricular cardiomyocytes because inhibition of AP-1 binding with decoy oligonucleotides blocked NO-induced apoptosis [1].

These examples show that AP-1 can serve as a mediator of either hypertrophy or apoptosis in one and the same cell type, i.e., in cardiomyocytes. This leads to the question, addressed in the present study, why AP-1 signaling may have these different outcomes.

One feature that influences the action of AP-1 is the composition of the AP-1 dimer itself. AP-1 is formed by dimerization of members of the bZIP protein superfamily. Jun proteins (c-Jun, JunB and JunD) can either homodimerize or form heterodimers with Fos family members [10]. All these homo- and heterodimers can bind to the AP-1 consensus binding site TRE as well as to variants of the TRE site. Their binding affinities to these various sites differ depending on the composition of the AP-1 complex [10,11]. Thus, there is a multitude of AP-1 dimers with different DNA binding specificities and different transactivating transcription potentials. As we have shown recently, the AP-1 dimer under hypertrophic stimulation of adult cardiomyocytes is a complex of c-Jun, JunB, c-Fos and FosB [2]. The composition of AP-1 under stimulation of apoptosis has not yet been investigated.

The action of AP-1 may also be modulated by additional transcription factors that interact with AP-1 and thereby change the pattern of AP-1 driven gene expression. Candidates are members of the SMAD and NFAT family. SMAD proteins are able to bind Jun family members directly [12]. This interaction results in synergistic transcriptional activity of SMAD proteins and AP-1, which has been shown to modulate transcription of several genes, i.e., of the endothelin-1 [13] or TGF\( \beta \) [14] genes. Proteins of the NFAT family are also able to bind promoter elements cooperatively with AP-1. Under these conditions, AP-1 binds to non-consensus sites in the promoter of various genes, i.e., of the IL-2 or Fas ligand gene [15], and enhances their expression only in the presence of NFAT. Therefore, different binding partners of AP-1 can modulate the promoter specificity and transcription-activating potential of AP-1, and as a result of this, they may change the outcome of AP-1 activation.

Because AP-1 has been identified as a mediator of hypertrophy and apoptosis in one and the same cell type, i.e., in ventricular cardiomyocytes of rat, it was the aim of the present study to analyze in this cell type (i) the composition of the AP-1 complexes under apoptotic conditions and (ii) the role of SMAD and NFAT transcription factors for the apoptotic or hypertrophic effect. Stimuli that are known to activate AP-1 in cardiomyocytes were used, i.e., the \( \alpha \)-adrenoeceptor agonist phenylephrine for hypertrophic growth stimulation and the NO donor SNAP for induction of apoptosis.

2. Methods

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.1. Cell isolation and cardiomyocyte cultures

Ventricular cardiomyocytes were isolated from 200 to 250 g male Wistar rats, suspended in basal culture medium and plated on culture dishes, which were preincubated overnight with 4% fetal calf serum in medium 199 as previously described [16]. The basal culture medium (CCT) was modified medium 199 including Earle’s salts, 2 mM L-carnitine, 5 mM taurine, 100 IU/ml penicillin, 100 \( \mu \)g/ml streptomycin and 10 \( \mu \)M cytosine-\( \beta \)-D-arabinofuranoside (pH 7.4). Three hours after plating, the dishes were washed twice with CCT medium. This results in cultures of about 90% quiescent rod-shaped cells on average.

2.2. Electrophoretic mobility shift assay (EMSA)

For generation of nuclear extracts, cardiomyocytes were homogenized in swelling buffer (10 mM Tris–HCl, pH 7.9, 10 mM KCl, 1 mM MgCl\(_2\), 1 mM DTT). After incubation for 1 h on ice, nuclei were pelleted by centrifugation at 900 rpm for 10 min. Pellets were homogenized in 10 mM Tris–HCl, pH 7.9, 300 mM sucrose, 1.5 mM MgCl\(_2\), 1 mM DTT, 0.3% triton X-100 and again centrifuged as above. These pellets were resuspended in storage buffer (10 mM HEPES, pH 7.5, 50 mM KCl, 300 mM NaCl, 1 mM EDTA, 1 mM DTT, 1 mM PMSF, 20% glycerol) on ice for 30 min and centrifuged at 13,000 rpm, 4 °C, for 5 min. The resulting supernatants were used in electrophoretic mobility shift assays (EMSA). Sequences of used oligonucleotides are given in Table 1. Complementary sequences of these oligonucleotides were hybridized. For radioactive labeling of oligonucleotides,
polynucleotide kinase and $\gamma^{32}$P-dATP was used. For fluorescence labeling, oligonucleotides were incubated with terminal transferase in the presence of Cy3-dCTP. After separation of unincorporated nucleotides from labeled oligonucleotides by gel filtration, oligonucleotides were incubated with 10 µl nuclear extracts in the presence of 1 µg poly(dIdC) at 30 °C for 30 min. The samples were run on 4% native polyacrylamide gels. Dried gels were exposed on phosphor imager (Molecular Dynamics) or fluorescence imager (BioRad). For identification of subunits in the binding complex, 0.5 µg antibodies were added to the reaction mixture prior to incubation of nuclear extracts with the oligonucleotides. This can either result in steric hindrance of binding, which causes a reduction of band shifts, or in bigger complexes and supershifts can appear. In our study, reductions in band shifts occurred. Antibodies mapped to the following epitopes: anti-c-Jun and anti-JunB within the amino terminal domain; anti-JunD and anti-a-Fos epitope at the carboxy terminus; anti-FosB within the central domain.

2.3. Transformation of cells with oligonucleotides

The last four bases at each end of oligonucleotides were phosphorothioate-modified to increase their stability in the mixture prior to incubation of nuclear extracts with the oligonucleotides. This can either result in steric hindrance of binding, which causes a reduction of band shifts, or in bigger complexes and supershifts can appear. In our study, reductions in band shifts occurred. Antibodies mapped to the following epitopes: anti-c-Jun and anti-JunB within the amino terminal domain; anti-JunD and anti-a-Fos epitope at the carboxy terminus; anti-FosB within the central domain.

2.4. Detection of chromatin condensation

Twenty four hours after apoptosis induction, cardiomyocytes were stained for 30 min with Hoechst 33258 (5 µg/ml) and propidium iodide (1 µg/ml). Hoechst 33258 is a membrane-permeable DNA dye that stains apoptotic, condensed nuclei more intensively. Propidium iodide only stains nuclei of necrotic cells, since it is unable to pass the cell membrane of intact or apoptotic cells. Cells were analyzed by fluorescence microscopy. For quantification of apoptosis and necrosis, 200 randomly distributed cells were counted in each experiment.

2.5. DNA laddering

Twenty four hours after agent addition, or in time-matched controls, DNA was extracted as described recently [20]. In brief, for DNA extraction cells were incubated in lysis buffer (100 mM NaCl, 10 mM Tris–Cl, 25 mM EDTA, 0.5% SDS and 100 µg/ml proteinase K, pH 8.0) for 3 h at 37 °C. DNA was extracted with phenol–chloroform and precipitated in ethanol. RNA was removed by digestion with DNase-free RNase (5 µg/ml) for 2 h at 37 °C. DNA of each sample (5 µg) was electrophoretically separated on agarose gels (1.5%) and stained with ethidium bromide.

2.6. Statistics

Data are given as means ± standard errors (S.E.) from n different culture preparations. Statistical comparisons were performed by one-way analysis of variance (ANOVA), and the Student–Newman–Keuls test for post hoc analysis was used [18]. A p-value of less than 0.05 was considered statistically significant. SPSS® software, version 11.5.1. (SAS Institute Inc., Cary, NC, USA), was used to analyze data.

2.7. Materials

Medium 199 was obtained from Boehringer (Mannheim, Germany), fetal calf serum from PAA (Linz, Austria), crude collagenase from Biochrom (Berlin, Germany), antibodies from Santa Cruz Biotechnology (Germany), oligonucleotides were from Invitrogen (Karlsruhe, Germany).

3. Results

3.1. Stimulation of JunB–FosB complexes by NO

Cardiomyocytes were incubated with the NO donor SNAP (100 µM) for stimulation of apoptosis. This
stimulation activated AP-1 binding within 2 h. The composition of the AP-1 dimer was analyzed in EMSAs by use of antibodies specific for Jun and Fos family members. As depicted in Fig. 1, panselective antibodies for all family members of either Jun or Fos reduced the AP-1 shift (Fig. 1). When using antibodies specific for only one Jun or Fos family member, c-Jun, JunB, and Fos B antibodies inhibited AP-1 binding stimulated by SNAP (Fig. 1). All other antibodies specific for Jun/Fos family members had no significant effects on AP-1 binding activity. These findings render unlikely the hypothesis that the outcome of AP-1 activation, resulting in either hypertrophy or apoptosis, depends on differences in composition of the AP-1 complex.

3.2. Induction of SMAD and NFAT during stimulation with NO and PE

NFAT or SMAD binding activity was analyzed in nuclear extracts of cardiomyocytes, which were stimulated either with SNAP for apoptosis induction or with the α-adrenoceptor agonist phenylephrine (PE, 10 μM) for stimulation of hypertrophic growth. Binding activity of transcription factors that may interact with AP-1 was found to be activated. Under both conditions, NFAT binding activity was activated: SNAP increased NFAT binding to 135.9 ± 6.1% and PE to 118.1 ± 5.7% of control (Fig. 2, p<0.05 vs. unstimulated controls, n=9). In contrast, SMAD binding was only activated by SNAP to 130.9 ± 9.6% of control (Fig. 2, n=9, p<0.05 vs. unstimulated controls) whereas incubation with PE even reduced SMAD binding activity (73.9 ± 13.6% of control, n=9, p<0.05 vs. unstimulated controls). The exclusive presence of SMAD proteins under apoptotic conditions makes them prime candidates that may...
interact with AP-1 in order to synergistically stimulate apoptosis. Therefore, composition and functional importance of this transcription factor was analyzed in further detail. Binding of SMAD proteins in EMSAs was confirmed by use of antibodies. As shown in Fig. 3, antibodies panselective for SMAD family members as well as antibodies specific for SMAD 4 and SMAD 3 inhibited binding of SMAD to its consensus binding site (106.8 ± 4.1%, 103.3 ± 3.5% or 105.6 ± 3.1% of control, n.s. vs. controls, n = 13), whereas antibodies specific for SMAD 1/5, SMAD 2 or Nrl, a transcription factor that is not related to the SMAD family, had no influence on SMAD binding induced by SNAP (120.3 ± 7.2%, 128.4 ± 14.4% or 130.2 ± 7.5% of control, p < 0.05 vs. control, n = 13). Therefore, during stimulation with SNAP, the binding activity of isoforms SMAD 3 and 4 is enhanced.

In order to test if an interaction between the transcription factors SMAD and AP-1 exists in nuclear extracts of SNAP-induced cardiomyocytes, nuclear extracts were preincubated with unlabeled oligonucleotides containing either binding sites specific for SMAD or AP-1 or with an oligonucleotide with no binding site. SNAP increased the binding of SMAD to its consensus binding site to 120.7 ± 5.7% (p < 0.05 vs. unstimulated controls, n = 4) (Fig. 4). While the scrambled oligonucleotide had no influence on this binding activity, both oligonucleotides containing either the SMAD or AP-1 binding site abolished SMAD binding in the EMSA. This indicates that an interaction between AP-1 and SMAD exists, since scavenging of AP-1 in the nuclear extracts abolished SMAD binding.

3.3. Involvement of SMAD in NO-induced apoptosis

The finding that SMAD is activated after incubation of cardiomyocytes with SNAP and not with PE suggests a role of SMAD in NO-stimulated apoptosis. In order to test this hypothesis, we used SMAD decoy oligonucleotides that inhibit SMAD binding activity intracellularly. After a 24-h incubation with SNAP, the number of apoptotic cells increased to 12.4 ± 1.2% of cardiomyocytes (vs. 5.9 ± 0.5% in unstimulated controls, p < 0.05, n = 5). When cardiomyocytes were transformed with SMAD decoy oligonucleotides, which inhibit SMAD binding activity intracellularly (Fig. 5), SNAP did not stimulate apoptosis.
in the cells (8.0 ± 1.0% apoptotic cells, n.s. vs. unstimulated controls, n = 5) (Fig. 6). Decoy oligonucleotides with a mutant SMAD binding site that did not inhibit SNAP-induced SMAD binding (Fig. 5) did not inhibit stimulation of apoptosis by SNAP (13.3 ± 0.9% apoptotic cells, p < 0.05 vs. unstimulated controls, n = 5). To test if the apoptotic response could be blocked when translation of SMAD4 is inhibited, we performed antisense studies. Cardiomyocytes were transformed with antisense oligonucleotides complementary to the translation start site of SMAD4 mRNA. Under these conditions, SNAP did not activate SMAD binding (Fig. 5) and could not induce apoptosis in the cells (8.0 ± 1.1% apoptotic cells, n.s. vs. unstimulated controls, n = 6) (Fig. 7). When cardiomyocytes were transformed with random oligonucleotides, SNAP still stimulated apoptosis (14.5 ± 1.8% apoptotic cells, p < 0.05 vs. unstimulated controls, n = 6). This identifies SMAD proteins, with SMAD4 as a central isoform in the complex, as mediators of NO-induced apoptosis in cardiomyocytes.

3.4. TGFβ1 induces apoptosis via AP-1 and SMAD signaling

TGFβ is a classic stimulator of SMAD proteins. Therefore, we tested if TGFβ induces SMAD in cardiomyocytes and if this is related to apoptosis induction. For stimulation of transcription factor binding activity, cardiomyocytes were incubated with 1 ng/ml TGFβ1 for 2 h. During this time, SMAD binding activity increased to 115.0 ± 3.1% and AP-1 was also activated to 123.6 ± 6.7% (p < 0.05 vs. unstimulated controls, n = 5) (Fig. 8). Binding activity of either SMAD or AP-1 could be blocked by transformation of myocytes with decoy oligonucleotides with specific binding sites for these proteins, whereas mutant oligonucleotides did not inhibit SMAD or AP-1 binding activity induced by TGFβ1 (Fig. 8). TGFβ also
increased the number of apoptotic cardiomyocytes: the appearance of a higher percentage of round cells with condensed nuclei could be seen during stimulation with TGFβ1, whereas in the controls and during inhibition of SMAD signalling by decoy oligonucleotides, the majority of the cells were rod-shaped and had non-condensed oval nuclei (Fig. 9C). Quantification of apoptotic cells revealed an increase from 12.7 ± 1.6% under control conditions to 21.7 ± 1.9% (p < 0.05, n = 6) (Fig. 9A). This apoptosis induction was blocked either by intracellular scavenging of SMAD proteins or AP-1: in cardiomyocytes treated with SMAD decoy oligonucleotides, 8.3 ± 0.9% apoptotic cells were found after incubation with TGFβ1, and in AP-1 decoy oligonucleotide-treated cells, 10.4 ± 0.9% apoptotic cells were found (n.s. vs. unstimulated controls, n = 10). Control oligos with mutant binding sites did not block TGFβ1-induced apoptosis (Fig. 9A). More evidence for induction of apoptosis by TGFβ1 was obtained from DNA laddering (Fig. 9B). Thus, for apoptosis, specific cleavage of DNA did not occur when SMAD activity was blocked by transformation of cardiomyocytes with SMAD or AP-1 decoy oligonucleotides. This confirms the findings of apoptosis induction via AP-1 and SMAD signaling by TGFβ1.

4. Discussion

The main finding of this study is the identification of the transcription factor SMAD as a mediator of apoptosis in ventricular cardiomyocytes. We demonstrate that SMAD
proteins are involved in NO- and TGFβ1-induced apoptosis. Under both conditions, simultaneous activation of the transcription factor AP-1 is shown and involvement of AP-1 in the process of apoptosis is also demonstrated. Therefore, interaction of AP-1 with SMAD proteins may direct AP-1 activity down a pro-apoptotic pathway.

AP-1 has been identified as a mediator of hypertrophic growth and apoptosis in ventricular cardiomyocytes. There are two possibilities to explain this different outcome of AP-1 activation: (i) target genes of AP-1 can differ, depending on the composition of the AP-1 dimer, or (ii) simultaneous activation of transcription factors, which can interact with AP-1, may be responsible for its apoptotic or hypertrophic action. To analyze which of these mechanisms contributes to AP-1-mediated hypertrophy or apoptosis, cardiomyocytes were stimulated with phenylephrine or SNAP, respectively. Phenylephrine is an α-adrenoceptor agonist and induces hypertrophic growth via PKC/IP3 signaling [19]. The NO donor SNAP mediates its apoptotic effects via the NO/cGMP pathway [20]. AP-1 has been shown recently, and also in this study, to be involved in both pathways [1,2,6].

In a first approach, we analyzed whether AP-1 composition differs under hypertrophic and apoptotic conditions. Using specific antibodies against different Jun and Fos family members, we detected a significant reduction of AP-1 binding activity in EMSAs with antibodies directed against c-Jun, JunB, and FosB under both conditions. Antibodies against other Fos and Jun family members had no effect on AP-1 binding under both conditions. This indicates that these Jun/Fos family members form the AP-1 complex under hypertrophic and apoptotic conditions. However, we cannot exclude the possibility that a small portion of the AP-1 complex may have other subunits that were not detected, because sensitivity of the assay may not be high enough. In spite of this, we can conclude that the main components of AP-1 induced by SNAP or phenylephrine are similar. At first glance, it may seem surprising that such different stimuli activate similar AP-1 complexes that are then responsible for stimulation of hypertrophy or apoptosis. However, JunB and FosB have been described also in other cell types as mediators of growth stimulation or apoptosis, i.e., in growth promotion by serum stimulation [21], or in apoptosis induction by withdrawal of erythropoietin [22], or in induction of apoptosis by TGFβ [23]. These examples and our own findings in cardiomyocytes show that activation of similar AP-1 dimers, composed of JunB and FosB, can either result in cellular growth or apoptosis. This suggests that the composition of AP-1 itself is not decisive for its action as a mediator of hypertrophic growth or apoptosis.

These findings motivated us to analyze the induction of other transcription factors that are able to interact with AP-1. One factor we considered was NFAT, because NFAT activation has been shown to occur under hypertrophic growth stimulation [24] and is known to enhance AP-1 binding affinity via cooperative binding [25]. NFAT activation, however, was found not only under stimulation with PE but also with SNAP. This excludes the possibility that NFAT is a regulating factor directing AP-1 signaling selectively to hypertrophy or apoptosis. Therefore, functional involvement of NFAT in the apoptotic pathway was not further investigated.

Another factor known to widen the spectrum of AP-1-controlled transcription is SMAD. SMAD proteins bind DNA with low affinity, also at their consensus binding sites. Multimeric repeats of the consensus binding motif or cofactors like AP-1 are needed to enhance SMAD binding [26]. Because of this weak binding affinity, we used a multimeric SMAD binding site in our EMSAs for detection of SMAD activation. Using these oligonucleotides, we detected SMAD activation during stimulation with NO but not with PE. Evidence for cooperation of SMAD/AP-1 binding during stimulation with NO came from the finding that inhibition of AP-1 binding activity abolished SMAD binding activity in nuclear extracts of the cells. The exclusive induction of SMAD during NO stimulation and its cooperative binding with AP-1 made SMAD a promising candidate as a signaling molecule in NO-induced apoptosis. In general, SMAD proteins bind to DNA as a complex of SMAD4 plus other SMAD family members [27]. Using specific antibodies, we detected SMAD4 and SMAD3 in the binding complex induced by NO in cardiomyocytes. The causal role of SMAD proteins in apoptosis induction was demonstrated by inhibition of SMAD binding with decoy oligonucleotides or suppression of SMAD4 synthesis with antisense oligonucleotides. Both interventions in SMAD signaling prevented induction of apoptosis by NO. Although the synergistic binding of AP-1 and SMAD to DNA is well known, to date there are only a few genes identified that are activated by both factors together. Concerning pro-apoptotic gene expression, enhancement of p53 and GADD45b mRNA is mediated by SMAD [28,29]. Since the minimal promoter of GADD45b does not show any identifiable SMAD binding site, additional factors like AP-1 may be needed for enhanced GADD45 transcription. However, evidence for AP-1 involvement has not yet been reported. Therefore, regulation of AP-1/SMAD-regulated and apoptotic-linked genes remains to be elucidated.

To investigate if SMAD proteins are common signaling molecules in cardiomyocyte apoptosis, we used TGFβ, a known stimulator of SMAD proteins. We demonstrate that TGFβ1 not only induces SMAD but also AP-1 in cardiomyocytes and that both molecules are needed for apoptosis induction by TGFβ1. TGFβ is an important signaling molecule in the heart. It is found to be elevated after myocardial infarction [30] and is known to contribute to the remodeling process by enhancement of cardiac fibrosis [31]. Our new findings in this study now indicate that, in addition to its fibrotic action, TGFβ may enhance the unfavorable remodeling process by apoptosis induction. Interestingly, elevation of SMAD proteins [32] and AP-1...
[8] are found in the chronic phase of myocardial infarct scar healing. Therefore, a pathophysiologic relevance of SMAD/AP-1 signaling after myocardial infarction can be assumed.

In conclusion, under apoptotic conditions SMAD proteins have been identified to be activated simultaneously with AP-1, and SMAD4 was shown for the first time to be involved in cardiomyocyte apoptosis. Under hypertrophic conditions, only AP-1 is activated and necessary for growth stimulation. Thus, SMAD proteins can be considered as potential candidates for proteins that shift AP-1 signaling from hypertrophy to apoptosis.

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