Identification of a specific target sequence for p53 family in the Jagged2 gene

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ABSTRACT

Inactivation of the tumour suppressor p53 is the most common defect in cancer cells. The p53 family members, p73 and p63, have remarkable structural similarities with p53 and can activate transcription of p53-responsive genes. In contrast to p53 however, p73 and p63 are rarely mutated in human cancers. Mice that lack p53 are developmentally normal, while p73 and p63 appear to play critical roles in normal development. In our earlier study, we showed that both p73 and p63 regulate the ligands for the Notch receptor, Jagged1 (JAG1) and Jagged2 (JAG2). We also identified a p63-binding site in the second intron of the human JAG1 gene. Here, we have analyzed the responsive element within the human JAG2 gene for p73/p63-dependent induction of gene expression. Using a chromatin immunoprecipitation assay, we identified a p73/p63-binding site located in the second intron of the JAG2 gene. A heterologous reporter assay revealed that this binding site is a functional response element and is relatively specific for p73 and p63 among the p53 family. This binding site consists of four copies of 10-bp consensus p53-binding motif and is highly conserved between human and rodents. Furthermore, both JAG1 and JAG2 mRNA were specifically up-regulated by either p63 or p73, but not by p53 in mouse embryo fibroblasts. Our findings demonstrate that JAG2 is indeed a direct and evolutionarily conserved transcriptional target of p73 and p63, and suggest a potential molecular mechanism for the involvement of the p53 family genes in normal development.

Key words : p53 family, p73, p63, Jagged1, Jagged2, Notch

INTRODUCTION

The p53 tumor suppressor is the most frequent target gene for genetic alterations in human cancers. p53 protein is present at low levels in normal cells but is activated in response to environmental stimuli such as DNA damage, hypoxia, viral infection or oncogene activation, resulting in transactivation of a specific set of

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target genes. These targets are involved in cell-cycle control, apoptosis, DNA replication, repair, proliferation, inhibition of angiogenesis and cellular stress response[1-3] The p53 family members p73 and p63 share high homology to p53, especially in the central DNA binding domain [4-6]. Thus, p73 and p63 can bind to the p53 responsive elements and up-regulate some p53-target genes, which suggest that the p53 family members have a potential for functional overlap with p53 itself [7-10].

Despite the structural and partial functional similarities, the p53 family members exhibit markedly divergent biological functions (for reviews, see refs 11). The majority of p53 mutations found in human cancers are clustered in the DNA binding domain, which leads to loss of sequence-specific DNA binding and transactivation, and ultimately inactivation of p53 function[12]. Unlike p53, p73 and p63 are rarely mutated in human cancers, suggesting that the two relatives are not classical tumor suppressor genes [11]. Additionally, p73 and p63 exert more pronounced effects on differentiation and development than p53. p53-deficient mice develop normally, but undergo spontaneous tumor formation [13]. In contrast, p73-deficient mice have neurological, pheromonal and inflammatory defects, but show no increased susceptibility to spontaneous tumorigenesis [14]. p63-deficient mice have major defects in their limbs and craniofacial development, as well as a striking absence of stratified epithelia, suggesting that p63 is required for limb and epidermal morphogenesis. In humans, Li-Fraumeni syndrome patients have inherited mutations of the p53 gene and develop normally, but are predisposed to cancer[15], while heterozygous germline mutations in the p63 gene are the cause of Ectrodactyly, Ectodermal dysplasia and facial Clefts (EEC) syndrome [16]. These differences are likely to depend on activation or repression of different sets of target genes.

In the context of this background, we performed cDNA microarray analysis of A172 human glioma cells overexpressed separately with p53, p73β and p63γ and identified the JAG1 and JAG2 genes as targets for p63 and p73 [17]. We here report a conserved responsive element for p73 and p63 (RE-JAG2) located within the second intron of the JAG2 gene. RE-JAG2 consists of four copies of the consensus 10-bp motif separated by 0, 2 and 3-bp, respectively. Thus, the p53 family proteins may recognize similar but also subtly different DNA sequences.

MATERIALS AND METHODS

Cell lines and recombinant adenovirus

The human cancer cell lines used in this study were purchased from American Type Culture Collection or the Japanese Collection of Research Bioresources (Osaka, Japan). All cell lines were cultured under conditions recommended by their respective depositors. The endogenous p53 statuses in these lines are wild type for A172, mutant for CHC4, PLC/PRF5, and DLD1, and p53 null for H1299. Mouse embryonic fibroblasts (MEF) were prepared according to standard methods [18] from day 9.5 embryos and were maintained in DMEM containing 10% fetal calf serum. The generation and purification of replication-deficient recombinant adenoviruses containing p53, p73β, and p63γ genes or the bacterial lacZ gene were described previously [19].

Northern blot analysis and semiquantitative RT-PCR

For Northern blot analysis, total RNA (10 μg) was electrophoretically separated on a 1% agarose gel containing 2.2 M formaldehyde and blotted on a nitrocellulose membrane (Schleicher & Schuell). RNA was visualized with ethidium bromide to ensure that it was intact and loaded in similar amounts and to confirm proper transfer. Hybridization was performed as described previously [17]. cDNA probes for human JAG1 (nucleotides (nt) 3531-4534), JAG2 (nt 419-1323), and p21 (nt 11-429) were amplified by the RT-PCR from appropriate cDNA pools. PCR products were sequenced to verify their identity. For semiquantitative RT-PCR analysis, cDNAs

were synthesized from 5μg total RNAs with SuperScript Preamplification System (Invitrogen). The RT-PCR exponential phase was determined on 20-30 cycles to allow semiquantitative comparisons among cDNAs from identical reactions. The PCR conditions involved an initial denaturation step at 94℃ for 2 min, followed by 30 cycles (for mouse Jag1 and Jag2) or 25 cycles (for mouse Gapdh) at 94℃ for 30 sec, 58℃ for 30 sec, and 72℃ for 1 min. Oligonucleotide primer sequences were as follows: mouse Jag1 forward 5'-AGTGCCAGTCTTCCCCTTGTG-3', mouse Jag1 reverse 5'-TCCAGTTCGGGTGTTTTGT CG-3', mouse Jag2 forward 5'-TGGGAGTTCCT GGATGGAAGA-3', mouse Jag2 reverse 5'-ACG GGCACCAACAGACCTGT-3', mouseGapdh forward 5'-ACCACAGTCCATGCCATCAC-3', and mouse Gapdh reverse 5'-TCCACCACCCTGTT GCTGTA-3'. The PCR products were visualized by electrophoresis on 1.5% agarose gels.

cDNA microarray

For cDNA expression arrays, poly(A)+ RNA was isolated with the FastTrack 2.0 mRNA isolation system (Invitrogen) from adenovirus-infected A172 human glioma cells and used as a template for synthesis of Cy3- or Cy5 -labeled cDNA probes. The probes were hybridized to cDNA microarrays containing 9216 genes. Microarray construction, hybridization procedures, and data analysis were described previously [20].

Chromatin immunoprecipitation assay (ChIP)

ChIP assay was performed using the Acetyl-Histone H3 ChIP Assay Kit (Upstate Biotechnology) as recommended by the manufacturer except that antibodies against p53 (DO -7, Santa Cruz Biotechnology), p63 (4A4, Oncogene Research), and FLAG tag peptide (M2, Sigma-Aldrich) were used in this study. 2×10^6 H1299 cells were plated onto a 10-cm dish and infected with Ad-p53 Ad-p73β or Ad-p63γ. After 24 h, genomic DNA and protein were cross-linked by addition of formaldehyde (1% final concentration) directly to culture medium and incubated for 15 min at 37℃. Cells were lysed in 200μl of SDS lysis buffer with a protease inhibitor mixture and sonicated to generate 300-800 bp DNA fragments. After centrifugation, the cleared supernatant was diluted 10-fold with the ChIP dilution buffer and incubated with the specific antibody at 4℃ for 16 h. Immune complexes were precipitated, washed, and eluted as recommended. DNA-protein cross-links were reversed by heating at 65℃ for 5 h, and DNA was phenol-extracted, ethanol-precipitated, and resuspended in 50μl of TE. Five microliters of each sample were used as a template for PCR amplification. PCR amplifications of the second intron of the JAG2 gene containing the consensus p53-binding sequences (+2020, renamed the RE-JAG2 sequence) were performed on immunoprecipitated chromatin using the specific primers 5'-CCCGCCGAGTATCCCTTGA-3' (forward) and 5'-CTGCTGCCAACTCGAGCA GA-3' (reverse). PCR amplification of the human MDM2 promoter containing a p53-binding sequence was performed using oligonucleotides 5'-GTTCAGTGGGCAGGTTGACT-3' (forward) and 5'-GCTACAAGCAAGTCGGTGCT-3' (reverse), as described [21]. To ensure that PCR was performed in linear range, template DNA was amplified for a maximum of 30 cycles. PCR products following ChIP assay were sequenced to verify the identity of the amplified DNA.

Luciferase assay

A 51-bp fragment of the RE-JAG2 (5'-(5'-AGGCAGGTCTGAGCAGGGCCCGCAGCCTGT CATCTGCACCTGGGC-3') was synthesized and inserted upstream of a basal SV40 promoter in the pGL3-promoter vector (Promega), and the resulting constructs were designated pGL3-RE-JAG2. A non-responsive control reporter plasmid, pGL3-p53CBSmut was also used. 1×106 of H1299 or HCT116 cells in 6-cm dishes were co-transfected with 1.5μg of either pGL3-RE-JAG2 or pGL3-p53CBSmut, together with 1.5μg of a pcDNA3.1 control vector (Invitrogen) or a vector that expresses p53, p73β, or p63γ using Lipofectin (Invitrogen). Cells were harvested 48

h after transfection followed by measurement of luciferase activity using the Luciferase Assay System (Promega). The ability to stimulate transcription was defined as the ratio of luciferase activity in the cells transfected with the pGL3-RE-JAG2 relative to the activity in the cells transfected with the non-responsive reporter plasmid, pGL3-p53CBSmut. All experiments were performed in triplicate and repeated on at least three independent occasions.

RESULTS

p63 and p73 induce expression of the JAG1 and JAG2 genes

To express the p53 family genes in human cancer cell lines, we used the replication-deficient adenoviral vector harboring human p53 (Ad-p53), p73β (Ad-p73β) and p63γ (Ad-p63γ) genes. To determine the relative efficiency of adenovirus-mediated gene transfer, cells were infected with adenovirus containing the bacterial lacZ gene (Ad-lacZ). We used five human cancer cell lines that showed highly efficient gene transfer, with 90 to 100% of the cells staining for β-galactosidase activity at a multiplicity of infection (MOI) of 50 to 100. A high-level of p53 protein was observed in cells infected with Ad-p53. Infection with Ad-p73β and Ad-p63γ resulted in expression of exogenous p73β and p63γ proteins, respectively (data not shown and refs) [17, 19, 21]. We used p73β and p63γ isoforms in this study, because we and others have demonstrated that transcription of a p53 responsive reporter gene was activated more strongly in p73β and p63γ than in p73α and p63α [5, 22]. In an effort to identify specific targets regulated by p73 and p63, we performed cDNA microarray analysis and compared expression patterns in human glioma cell line A172 transfected separately with Ad-p53, Ad-p73β and Ad-p63γ. Using this approach, we identified the JAG1 and JAG2 genes as targets for p63 and p73 [17]. Using the JAG1 and JAG2 cDNAs as probes, we performed Northern blot analysis with five human cancer cell lines, including A172, CHC4 and PLC/PRF5 (hepatocellular carcinoma), DLD1 (colorectal cancers), and H1299 (lung cancer) (Fig. 1). JAG1 was highly induced by p63γ and by p73β in four of the five lines tested (no induction in PLC/PRF5 cells). In contrast, JAG1 induction by p53 was less dramatic, and occurred in only two cell lines (A172 and DLD1 cells). JAG1 expression was reduced by p53 in PLC/PRF5 and H 1299. On the other hand, JAG2 was highly induced by p63γ and by p73β in all human cancer cell lines tested. Although a similar induction was seen in Ad-p53-infected H1299 cells, JAG2 induction by p53 was considerably less in the other cell lines (Fig. 1, second panel). Moreover, the strongest induction of JAG2 was observed

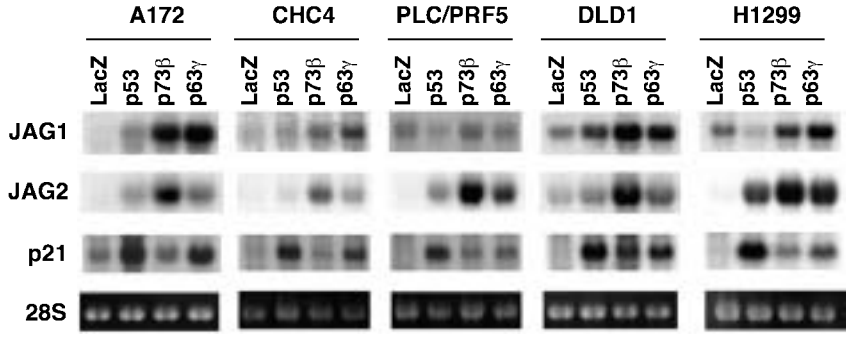

Fig. 1 Northern blot analysis shows JAG1 and JAG2 induction in human cancer cell lines
Five human cancer cell lines were infected with adenoviruses at a MOI of 50 or 100, and the cells were harvested 24 h after infection. Total RNA were extracted and subjected to Northern blotting. Total RNA (10 μg) were loaded in each lane, and the same filter was re-hybridized with human JAG1, JAG2 and p21 cDNAs. Ethidium bromide staining of 28S ribosomal RNA (28S) in the lower panel shows that equal amounts of RNA were loaded in each lane.

following Ad-p73β infection in all cell lines (Fig. 1). Ad-p53 induction of p21 was observed in all five lines, but Ad-p63γ and Ad-p73β induced p21 only in a subset of these lines (Fig. 1, third panel). Taken together, the results presented here suggest that the JAG1 and JAG2 genes are specific targets of p73 and p63.

Identification of a specific target sequence for p73 and p63 in the JAG2 gene

The strong induction of JAG2 suggests that JAG2 may be a direct and specific target of transcriptional activation by p73 and p63. To address this hypothesis, we searched for a consensus p53-binding sequence in the JAG2 gene, because the p73 and p63 proteins can also bind to the p53-binding sites [4-7]. We obtained the genomic sequence of the human JAG2 gene from the GenBank database (Accession Number: NT026437) and searched for a consensus p53-binding site(s) within 10 kb in and around exon 1 of the JAG2 gene. Eight candidate sequences were identified at the positions −4353, −368, +993, +1017, +2020, +2487, +5033 and +5951, where +1 represents the translation initiation site. We then performed ChIP assay to determine whether the p63 and p73 protein could bind to these candidate sequences in vivo. The ChIP assay relies on the ability of specific antibodies to immunoprecipitate DNA-binding proteins along with the associated genomic DNA (Fig. 2b). We used H1299 cells infected with Ad-p53, Ad-p73β, and Ad-p63γ. Immunoprecipitation of DNA-protein complex using antibodies against p53, p73, and p63 was performed on formaldehyde-crosslinked extract from Ad-p53-, Ad-p73β-, and Ad-p63γ-infected H1299 cells, respectively. We then measured the abundance of candidate sequences within the immunoprecipitated complexes by PCR. As shown in Figure 2c, one DNA fragment containing a candidate sequence at +2020 was reproducibly present in the immunoprecipitated complex containing p73 or p63 protein (top row, lane 8 and 11). We designated this sequence RE-JAG2 (for responsive element in JAG2), which consists of four copies of the consensus 10-bp motif separated by 0, 2 and 3-bp, respectively (Fig. 2a). In contrast, we did not detect p53 protein binding to the RE-JAG2 sequence in Ad-p53-infected cells, as assessed by the ChIP assay and subsequent PCR amplification (top row, lane 5). The other candidate sequences were amplified in the chromatin input control but not in the samples immunoprecipitated with an antibody against p53, p73, or p63 (data not shown). As a positive control for the ChIP assay, we analyzed the interaction of p53 family proteins with the MDM2 promoter. As expected, p53, p73 and p63 proteins immunoprecipitated with the DNA fragment containing the p53-binding site in the MDM2 promoter (Fig. 2c, bottom row, lanes 5, 8 and 11). Thus, these results indicated that both p73 and p63 proteins can bind specifically to RE-JAG2 in H1299 cells in vivo.

To determine whether p73 and p63 can transactivate gene expression via RE-JAG2, we performed a heterologous promoter-reporter assay using a pGL3-RE-JAG2 luciferase vector that contains RE-JAG2 sequence upstream of the basal SV40 promoter (see Materials and Methods). H1299 and HCT116 cells were transiently co-transfected with pGL3-RE-JAG2 together with a p53, p73β, or p63γ-expressing plasmid. Fig. 3c shows that luciferase activity from pGL3-RE-JAG2 is higher in cells co-transfected with either p73β or p63γ than in those co-transfected with p53. These results are consistent with the induction of endogenous JAG2 by p73 and p63 in several cancer cells (Fig. 1). Together, these results also support the idea that RE-JAG2 can mediate p73/p63-dependent transcriptional activation, leading to the conclusion that JAG2 is a direct target of p73 and p63.

The p73/p63-binding site is highly conserved between human and rodents JAG2 genes

If the JAG1 and JAG2 genes have important roles in the downstream effect of p53 family, their activation by p73 or p63 would be conserved among species. We thus performed sequence comparison of the p73/p63 binding site

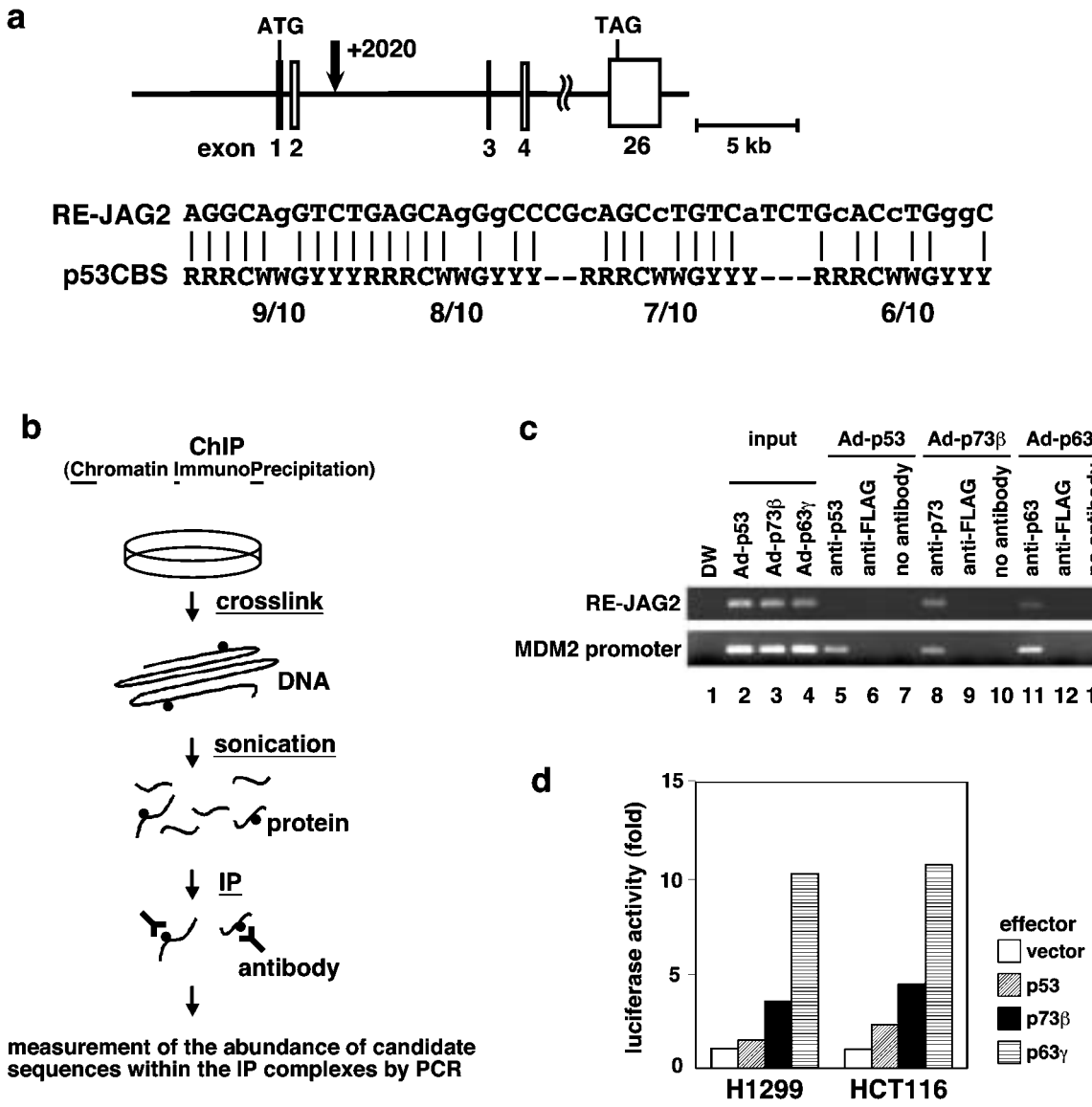

Fig. 2 The p63 and p73 response element in human JAG2 gene

(a) The position and sequence of the responsive element for p73 and p63, RE-JAG2 are shown. Open boxes represent exons. RE-JAG2 is located in the second intron of the JAG2 gene and consists of four copies of the consensus 10-bp motif of the p53-binding sequence. The nucleotide sequences of RE-JAG2 are indicated by uppercase letters and consensus sequences are indicated by lowercase letters. The nucleotides that match with the consensus sequences for p53 binding are indicated with vertical lines. The spacer sequences between the 10-bp motifs are underlined. R represents purine; Y, pyrimidine; W, adenine or thymidine.

(b) Flowchart for the chromatin immunoprecipitation (ChIP) assay.

(c) p73 and p63 interacts with the RE-JAG2 sequence in vivo.
ChIP assay of a genomic fragment containing the RE-JAG2 sequence in Ad-p53-infected (lanes 5 to 7), Ad-p73β-infected (lanes 8 to 10), or Ad-p63γ-infected (lanes 11 to 13) H1299 cells is shown (upper panel). Immunoprecipitation was performed using the gene-specific antibody against p53 (lane 5), p73 (lane 8), or p63 (lane 11), followed by PCR amplification. Input chromatin represents a portion of the sonicated chromatin prior to immunoprecipitation (lanes 2 to 4). Immunoprecipitates with an anti-FLAG antibody (lanes 6, 9, and 12) or in the absence of antibody (no antibody, lanes 7, 10, and 13) were used for controls. DW indicates a no template control (lane 1). PCR amplification of the MDM2 promoter was per-

formed using primers that flank the p53-binding site in the MDM2 promoter. PCR amplification revealed that a similar amount of MDM2 promoter sequence was present in p53-, p73-, and p63-complexes extracted from each immunoprecipitate (lower panel). The RE-JAG2 was amplified in the immunoprecipitated samples with an antibody against p73 (upper panel, lane 8) and p63 (upper panel, lane 11).

(d) The RE-JAG2 in the JAG2 gene is responsive to p73β and p63γ. H1299 and HCT116 cells were co-transfected with either pGL3-RE-JAG2 or pGL3-p53CBSmut, together with a control pcDNA3.1 vector or a vector that expresses p53, p73β, or p63γ gene. Cells were harvested 48 h after transfection followed by measurement of luciferase activity using the Luciferase Assay System (Promega). The ability to stimulate transcription was defined as the ratio of luciferase activity in the cells transfected with the pGL3-RE-JAG2 relative to the activity in the cells transfected with the non-responsive reporter plasmid, pGL3-p53 CBSmut. All experiments were performed in triplicate and repeated on at least three independent occasions.

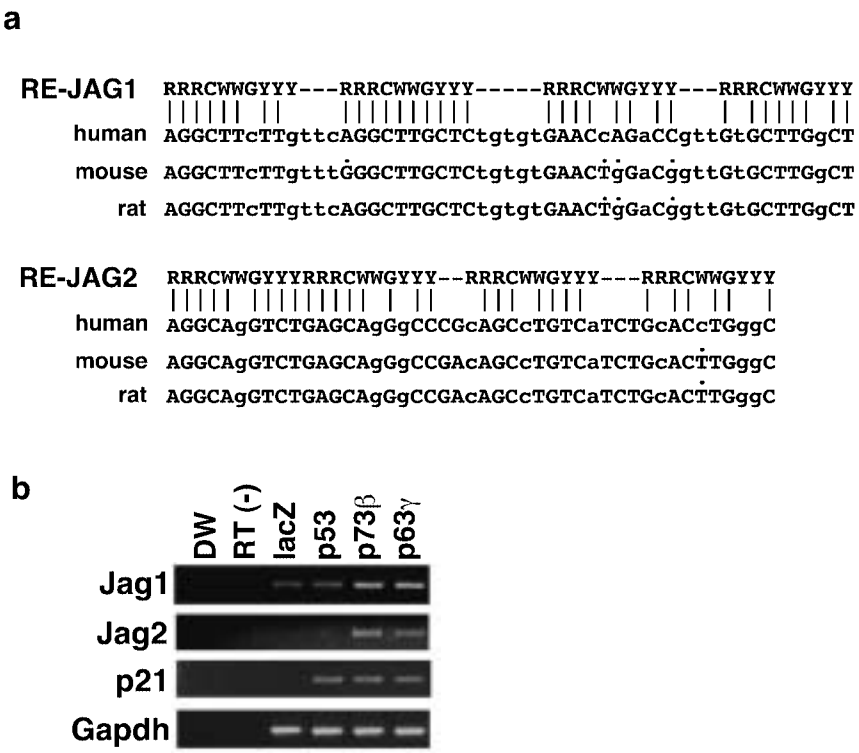

Fig. 3 The p73/p63-binding site located within JAG1 and JAG2 gene is highly conserved between human and rodents

(a) Alignment of conserved p73/p63 binding sites in human, mouse, and rat sequences from the JAG1 and JAG2 gene. Mismatched nucleotides are indicated by "·". Species and sequence accession numbers are as follows: human JAG1, NT011387; mouse Jag1, NT039207; rat Jag1, NW047658; human JAG2, NT026437; mouse Jag2, NT039551; rat Jag2, NW047762.

(b) Semiquantitative RT-PCR analysis showing Jag1 and Jag2 mRNA induction after Adenovirus-mediated transfer of the human p53 gene family in mouse cells. MEFs were infected with adenoviruses at a MOI of 100. Total RNAs were prepared from the cells at 24 h after infection. RT-PCR analysis was performed as described in Materials and Methods. Distilled water (DW) and total RNA untreated with reverse transcriptase (RT(-)) were used for negative controls. We also performed semiquantitative RT-PCR analysis of known p53-target gene, p21. Gapdh mRNA levels were examined as a control for integrity of the cDNA.

among mammalian JAG2 genes. Nucleotide sequence analysis has shown that the Jag2 genes in mouse and rat contained, in almost identical position, RE-JAG2 sequence (Fig. 3a). In our previous paper, we discovered that the human JAG1 gene contains a response element specific for p63 in its second intron, which consists of four copies of the consensus 10-bp motif separated by 3, 5 and 3-bp, respectively. This binding site, RE-JAG1, is also conserved in the mouse and rat Jag1 genes (Fig. 3a). Moreover, expression of Jag1 and Jag2 mRNA was increased in MEFs 24 h after infection with Ad-p73β or Ad-p63γ, but was not significantly increased after infection with Ad-p53 or Ad-LacZ, similarly to p21/WAF1 expression (Fig. 3 b). These results indicated that transcription of the Jag1 and Jag2 gene is regulated by both p73 and p63 in mouse cells.

DISCUSSION

The tumor suppressor p53 is critically important in the cellular damage response and is the founding member of a family of proteins. Two members of the mammalian p53 family, p73 and p63 encode proteins that share considerable structural homology with p53, suggesting that the p53 family genes have a potential for functional overlap. Indeed, the expression of several p53-regulated genes can also be induced by p73 and p63, though recent studies show a marked divergence in the developmental roles of p63 and p73, and further distinguished these p53 family genes from p53 [11]. Despite these revelations, little is known about target genes specifically regulated by p63 or p73. Thus, identifying the gene targets of p63 and p73 that play a role in development is an important step to gain a better understanding of the roles of these genes in normal development and developmental disorders. Previous studies from our laboratory demonstrated that induction of JAG1 and JAG2 is seen following ectopic expression of p63 or p73 in various types of human cancer cell lines regardless of p53 status [17]. We also identified a specific binding site for p63 in the second intron of JAG1. Here, we report the identification of a specific binding site for p73 and p63 in the second intron of the human JAG2 gene. The sequence comparison reveals that rodent Jag1 and Jag2 genes have conserved p73/p63 binding sites. Together with the present finding that both Jag1 and Jag2 mRNA is preferentially induced by p63 and p73 in MEF cells, we concluded that the JAG1 and JAG2 genes are direct and evolutionarily conserved transcriptional targets of p73 and p63.

The ability of p53 to transactivate genes containing specific binding motifs is central to its role as a tumor suppressor protein [1-3]. p73 and p63 can also bind to the p53 responsive elements and up-regulate some p53-target genes. We identified the specific binding site for p73 and p63, RE-JAG2 in the second intron of the JAG2 gene by a ChIP assay. A reporter assay demonstrated that RE-JAG2 was important for p63-dependent transactivation. RE-JAG2 consists of four copies of the 10-bp p53-binding motif separated by 0, 2 and 3-bp nucleotides, while nearly all p53 response elements reported previously contain two adjacent copies of the 10-bp p53-binding motif [1-3, 23]. Our laboratory and others have shown that response elements for p73 and/or p63 in their target genes including AQP3[24], the p73 gene itself [25], JAG1[17], IL-4Rα[21], and PEDF [26], consist of three or more copies of the 10-bp consensus p53-binding motif separated by spacer sequences. Therefore, spacing between at least three of the copies of the 10-bp motif may be important for determining the binding specificity of the p53 family member proteins.

To date, five Notch ligands have been identified, including JAG1, JAG2, Delta like-1 (DLL1), DLL3 and DLL4 [26-30]. In general, ligand binding induces intracellular cleavage and activation of Notch proteins [31-32]. The cleavage product translocates to the nucleus, in which it interacts with the transcription factor CBF1 to transactivate effector target genes, including hairy enhancer of split-1 (HES1)[31]. We have previously shown that p63-mediated JAG1 and JAG2 ex-

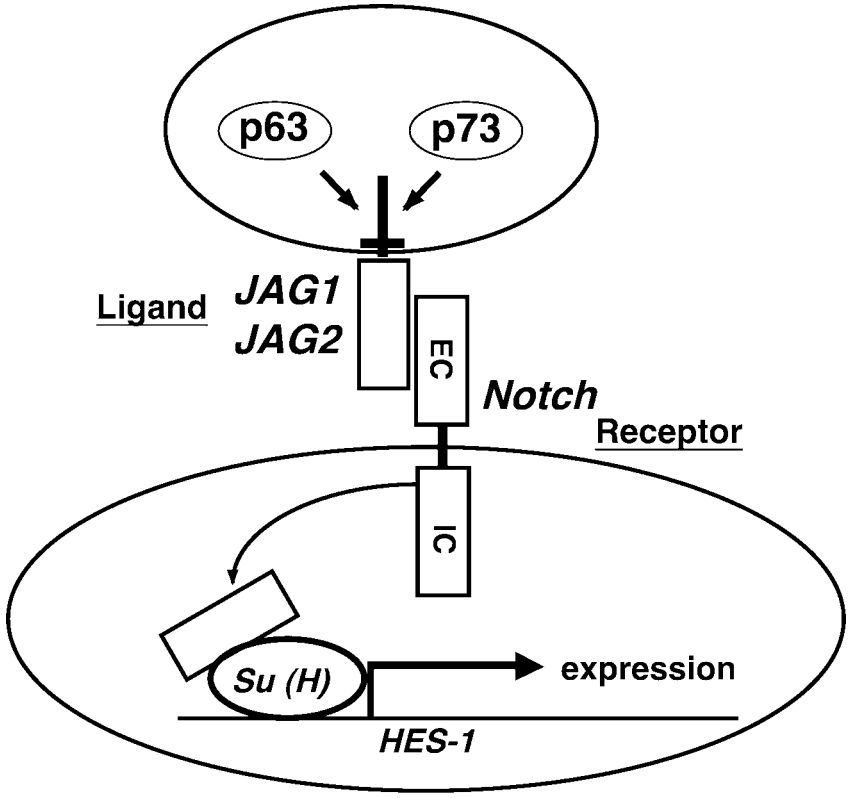

Fig. 4 Model of the functional relationship between p53 family and Notch signaling
IC, intracellular domain; EC, extracellular domain.

pression can transactivate endogenous HES-1 gene expression in co-cultured cells[17], suggesting that p63 can trigger the Notch signal pathway in neighboring cells (Fig. 4). Here we have shown that JAG1 and JAG2 activation by p73 or p63 is conserved between human and rodents, and this raises the possibility that the p53 family member genes play a role in normal development by modulating the Notch signal pathway.

During development, Notch signaling mediates many processes, including the segregation of neural and epidermal lineages [33-34]. Mutations in human Notch ligands result in the disruption of the Notch signal pathway, leading to developmental abnormalities[35-37]. Mutations in the JAG1 gene have been found in patients with Alagille syndrome (AGS), an autosomal dominant disorder characterized by abnormal development of heart, skeleton, liver and eye, as well as a characteristic facial appearance. Interestingly, JAG2 deficient mice exhibit defects of limb and craniofacial development [38], closely resembling the phenotype of patients with EEC syndrome, in which p63 is mutated. Both p63 and p73 can transactivate the promoters of genes associated with neuronal or epidermal differentiation and overexpression of these genes up-regulates neuronal or epidermal differentiation markers [39]. Together with our findings, these studies highlight the potential for an interplay between the p53 family genes and Notch signal pathway during ectodermal development.

In conclusion, we have characterized the specific binding site for p73 and p63, RE-JAG2 in the JAG2 gene, which is necessary and sufficient for direct regulation by p73 and p63, identifying JAG2 as a downstream target of p73 and p63. Moreover, rodent Jag2 genes also have RE-JAG2 site. Our results suggest that there are likely to be differences among the p53 family members with regard to their optimal DNA-binding sequences and that p73 and p63 play a role in normal development and cellular regulation mediated by Notch signaling.

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