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The retinal dehydrogenase/reductase *retSDR1/DHRS3* gene is activated by p53 and p63 but not by mutants derived from tumors or EEC/ADULT malformation syndromes

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Key words: p53/p63 proteins, retSDR1, DHRS3, vitamin A, development, tumor suppression, embryonal malformation, EEC syndrome, SHFM syndrome, ADULT syndrome

Abbreviations: retSDR1, retinal short-chain dehydrogenase/reductase 1; DHRS3, dehydrogenase/reductase member 3; EEC syndrome, ectrodactyly, ectodermal dysplasia and cleft lip/palate syndrome; SHFM syndrome, split hand/split foot malformation syndrome; ADULT syndrome, acro-dermato-ungual-tacrimal-tooth syndrome

Retinol and its metabolites have important roles in many processes including embryonic development, cellular differentiation, apoptosis and maintenance of epithelia. Retinal short-chain dehydrogenase/reductase retSDR1, also known as dehydrogenase/reductase member 3 (DHRS3), is involved in maintaining the cellular supply of retinol metabolites. We observe that *retSDR1* expression is activated by members of the p53 family. Particularly p53 and TAp63γ regulate transcription through two separate response elements in the *retSDR1* promoter. Both proteins bind the promoter in vitro and in vivo. Induction of DNA damage leads to recruitment of p53 and p63 to the *retSDR1* promoter. A tumor-derived p53 mutant is unable to activate *retSDR1* transcription. As mutants of *p63* in humans exhibit phenotypes that cause several autosomal dominantly inherited syndromes leading to developmental malformations, we tested the transcriptional response of TAp63γ mutants derived from the EEC, SHFM and ADULT syndromes. EEC syndrome-specific mutations of TAp63γ fail to transactivate *retSDR1* and an ADULT syndrome-derived mutant stimulates *retSDR1* transcription significantly less than the wild-type variant of p63. Taken together, the results suggest a potential role of the p53/p63-mediated *retSDR1* activation in tumor suppression as well as in developmental processes.

Introduction

Vitamin A (retinol) and its metabolites are essential for life and have important roles in embryonic development, cellular differentiation, apoptosis, vision, fertility, immune response and maintenance of various epithelia.[1-4] Several of theses functions may be related to stem cell renewal. There is a large body of evidence showing that retinoids control embryonic development through their interaction with retinoic acid nuclear receptors acting as central transcription factors.[3]

Many dehydrogenases regulate conversion of vitamin A derivatives.[5] Several of these enzymes yield a direct link from enzyme activity to functions in organ development.[6] Retinal short-chain dehydrogenase/reductase retSDR1, also known as dehydrogenase/reductase member 3 (DHRS3), is a member of the SDR family and was identified as an all-trans-retinol dehydrogenase in cone photoreceptors.[7,8] The retSDR1 enzyme observed in photoreceptor outer segments in the retina was suggested to be involved in the reduction of all-trans-retinal replenishing the retinoid reservoir which is rate limiting to the regeneration of bleached pigments in the visual cycle.[7] Interestingly, retSDR1 may play a more general role in retinol metabolism since its expression was observed in many fetal and adult tissues.[9] Additionally, expression of *retSDR1* was also detected in several neuroblastoma cell lines. Treatment of these cells with retinoids yields an increase in *retSDR1* expression. Induced retSDR1 expression after treatment of cells with retinaldehyde or retinol leads to accumulation of retinyl esters, which represent important storage forms of vitamin A metabolites to be ultimately used in all retinol functions.[9]

Some evidence links the *retSDR1* gene to cancer prevention. The gene is located on chromosome 1p35.1.[7] This region is frequently rearranged in human cancer and its deletion is associated with a poor prognosis. Monoallelic deletion of *retSDR1* in several neuroblastoma cell lines results in delayed accumulation of *retSDR1* mRNA expression in response to retinoids.[9] Furthermore, aberrant CpG-island methylation of the *retSDR1*

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Table 1. Stimulation of *retSDR1* mRNA expression after selective induction of p53 family members

Induction of	*retSDR1* mRNA fold change
TAp63γ	16.3
TAp63α	4.8
TA*p63α	nc
TA*p63γ	nc
ΔNp63α	nc
ΔNp63γ	nc
p53	44.3

Levels of *retSDR1* mRNA were measured by real-time RT-PCR from DLD-1 colorectal adenocarcinoma cells stably transfected with tet-off vectors expressing members of the p53 family. Changes in *retSDR1* mRNA expression are given as fold change comparing mRNA levels 9 h after induction to levels before induction. Standardization was performed by quantifying *GAPDH* mRNA expression and nc denotes no significant change.

promoter in human melanomas was observed, likely resulting in silencing gene transcription in cancer cells.[10]

Generally, alteration in vitamin A metabolism seems to be an important event in carcinogenesis. Various reports demonstrated that vitamin A deficiency leads to an increased development of spontaneous and chemically induced tumors.[11] Consistent with these observations, vitamin A supplementation to diets results in decreased chemically induced tumor incidence. Retinoic acid prevents tumor development by inhibition of proliferation,[12-14] stimulation of differentiation,[15] induction of apoptosis[16,17] or combinations of these mechanisms.

Transcriptional regulation by members of the p53 family contributes to the control of apoptosis, development and differentiation.[18] p53 and p63 proteins mediate activation of their target genes by binding specific recognition elements.[19] Members of the p53 family are characterized by high sequence similarity, particularly in the DNA-binding domain. This leads to a considerable overlap of target promoters, but also allows for recognition and transactivation of distinct target genes. p53 is a well characterized tumor suppressor.[20,21] However, it was recently discovered that p53 holds various other functions. Important examples are its roles in differentiation and embryonic development,[22,23] in glucose metabolism,[24] mediating expression of proteins forming intracellular structures or the extracellular matrix,[25-28] or in blastocyst implantation as observed in human pregnancy[29] or through LIF regulation in mice.[30]

p63 proteins seem to have minor functions in tumor suppression. Much more pronounced than p53, this protein family plays an important role in development and differentiation. In p63-deficient mice limbs are absent or truncated, the skin does not develop beyond an early stage, and hair follicles, teeth and mammary glands are absent.[31-33] Importantly, a study using ChIP-on-chip to screen for p63 target genes in keratinocyte cell systems identified many targets involved in development/morphogenesis and cancer.[34] Thus, p63 is essential for several aspects of epithelial development and morphogenesis.

Similar to the mouse model, heterozygous *p63* mutations in humans cause several syndromes leading to combinations of ectrodactyly, ectodermal dysplasia, cleft lip, split hand and foot malformations. The combination of malformations depends on which amino acid in the resulting p63 proteins are changed.[35-38] Many of the alterations in cell differentiation and general malformations seen in patients or mice lacking wild-type *p63* overlap with deficits observed in development due to insufficient retinol function.[1-3]

Here, we identify *retSDR1* as novel transcriptional target of the p53 family. The observations link tumor suppression and malformation syndromes to regulation of *retSDR1* by p53 family proteins.

Results

Induction of retSDR1 expression by members of the p53 family. In a DNA microarray screening employing the colorectal adenocarcinoma cell line DLD-1 carrying stably introduced TAp63γ transgenes, mRNA expressed from the retinal dehydrogenase *retSDR1* gene was observed to increase after induction of TAp63γ expression.[39,40] In order to test this preliminary observation, we applied real-time RT-PCR on similar samples. Consistent with the initial finding, *retSDR1* mRNA was found strongly upregulated after induction of TAp63γ protein expression (**Table 1**).

The p63 family members are expressed as several distinct variants.[41] Therefore, we tested the influence of various family members on *retSDR1* expression. Interestingly, selective p53 expression yielded a strong induction of *retSDR1* by more than 40-fold. Furthermore, we observed a significant increase of the *retSDR1* mRNA level after TAp63α induction. None of the other family members tested affects expression of *retSDR1* significantly (**Table 1**).

More importantly, retSDR1 protein expression is also induced when its mRNA level increases after induction of p53 family members. As a positive control, retSDR1 protein was detected from human colon cancer HCT116 cells transfected with a *retSDR1*-expression plasmid by western analysis (**Fig. 1**). A clear induction of retSDR1 protein was observed following the overexpression of p53. The induction of retSDR1 protein after TAp63γ expression appeared less pronounced, consistent with the lower mRNA induction compared to p53 (**Fig. 1**).

TAp63γ and p53 regulate transcription from the *retSDR1* promoter. The observation that p53 and TAp63γ significantly influence *retSDR1* expression led us to analyze transcriptional control from a 2 kb genomic DNA segment upstream of the translation *retSDR1* start codon. This fragment was amplified and cloned into a firefly luciferase-expressing reporter plasmid constituting the h-retSDR1-2000 construct (**Suppl. Fig. 1A**). Cotransfections of this construct with wild-type *p53* or *TAp63γ* expression plasmids into SaOS-2 cells cause significantly increased luciferase activity (**Fig. 2A**). In contrast, DNA-binding deficient mutants of p53 or TAp63γ are not able to enhance expression of the reporter. Both, expression of TAp63γ and p53 led to strong transcription from the *retSDR1* promoter construct over their DNA-binding mutants (**Fig. 2A**).

Two novel response elements mediate transcription of *retSDR1* by p53 and TAp63γ. In order to localize the position

of the responding element in the *retSDR1* promoter, deletion mutants were created and tested (**Suppl. Fig. 1A**). Essentially a loss of TAp63γ-mediated transactivation is observed after truncation of the promoter from the -1200 to the -650 bp construct. In contrast, p53 is still able to transactivate this promoter fragment (**Fig. 2B**). This narrows down potential activating regions and suggests that TAp63γ and p53 mediate transcriptional effects by two distinct sites.

In silico analysis of the promoter revealed two potential bindings sites which resemble the classical p53 consensus. The two sites were mutated on the basis of the -1400 bp construct (**Suppl. Fig. 1B**). Mutation of the downstream element proximal to the coding region (site 2) resulted in a clear reduction of p53-mediated transactivation compared to the wild-type promoter construct. In contrast, site 2 mutation affects TAp63γ-mediated activation only to a minor extent (**Fig. 2C**). p53-dependent reporter-gene activation was also strongly reduced when site 1 is altered. However, activation of *retSDR1* by TAp63γ is essentially abrogated for this mutant. Consistently, mutation of both consensus sites abolished activation by p53 as well as by TAp63γ (**Fig. 2C**). Furthermore, phylogenetic footprint analyses revealed a strong conservation of the two sites in vertebrates suggesting a biological relevance of the elements (data not shown).

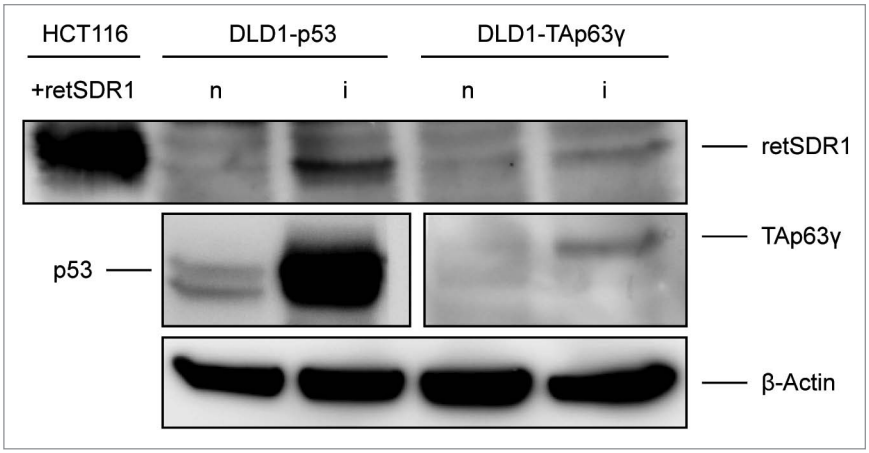

Figure 1. Stimulation of retSDR1 protein expression after induction of p53 and TAp63γ. Western analysis with HCT116 cell lysates (7 μg) transfected with a plasmid expressing retSDR1 served as a positive control (+retSDR1). RetSDR1 protein expression was compared before (n) and 9 h after (i) tet-off regulated p53 or TAp63γ expression in the colorectal adenocarcinoma cell line DLD-1. Fifty μg total cell lysate was loaded per lane. RetSDR1 protein was detected with a monoclonal antibody kindly provided by Françoise Haeseleer. Also, induction of p53 and TAp63γ expression was analyzed by comparison of cell lysates before (n) and after induction (i) of the transgenes. Detection of β-actin served as a loading control.

In summary, we identified two novel recognition elements in the *retSDR1* promoter which are responsible for transcriptional regulation by p53 and TAp63γ. p53 appears to activate *retSDR1* through both sites whereas TAp63γ-dependent activation is nearly exclusively conveyed by the upstream element site 1.

The upstream site controls *retSDR1* transactivation by several members of the p63 family. ΔNp63α, ΔNp63γ, TAp63α, TA*p63α and TA*p63γ, as additional members of the p63 family, were analyzed for their ability to activate the *retSDR1* reporter (**Fig. 2D**). TA*p63γ, a longer variant than the TAp63γ isoform, activated the reporter gene slightly. Whereas TAp63α hardly affected the transactivation of *retSDR1*. Significantly, all proteins tested failed to activate *retSDR1* transcription after site 1 was mutated (**Fig. 2D**). This supports the conclusion that site 1 is essential for regulation by p63 family members.

TAp63γ and p53 bind to the novel *retSDR1* response elements in vitro. TAp63γ or p53 proteins were synthesized by translation in vitro and tested in EMSAs for their binding to oligonucleotides representing site 1 from the *retSDR1* promoter. Both proteins shifted the probe and yielded a supershift when incubated with respective antibodies (**Fig. 3A**). Oligonucleotides from the *p21*$^{WAF1/CIP1}$ promoter served as positive controls. TAp63γ or p53 mutant proteins with alterations in their DNA binding domains were employed as negative controls (**Fig. 3A**). Furthermore, binding of p53 or TAp63γ was also impeded when site 1 was mutated in the *retSDR1* probe (**Fig. 3B**). Consistently, protein binding to oligonucleotides carrying the p53-binding site from the *mdm2* promoter was competed by wild-type *retSDR1*-site 1 oligonucleotides whereas mutant *retSDR1*-site 1 oligonucleotides failed to compete (**Fig. 3C**). Again, the assays confirmed specific binding of TAp63γ and p53 to site 1 from the *retSDR1* promoter.

Similar experiments employing a probe containing site 2 from the *retSDR1* promoter for binding of p53 or TAp63γ proteins were performed. Generally, a much weaker but specific binding to site 2 for both proteins was observed (data not shown).

TAp63γ and p53 proteins can bind to the *retSDR1* promoter in vivo. Binding in vivo was tested by chromatin immunoprecipitation (ChIP) analyses from DLD-1 cells after tet-off-induced expression of either TAp63γ or p53 proteins. For precipitation of p63 an antibody was employed that recognizes all p63γ isoforms. Analyzing the region of the *retSDR1* promoter carrying the identified novel binding element site 1 yielded a significant signal after induced expression of the *TAp63*γ transgene (**Fig. 4**, upper). Binding of TAp63γ was also detectable when primers specific for site 2 were utilized (**Fig. 4**, lower). Furthermore, also p53 is recruited to the *retSDR1* promoter. DNA fragments with site 1 and 2 were detected in ChIP experiments. Generally, although we tried to distinguish between site 1 and 2, one has to keep in mind that the ChIP method is not able unequivocally discriminate between bindings too closely placed sites in one promoter. Summarizing these observations, we could demonstrate binding of TAp63γ and p53 to the *retSDR1* promoter in vivo.

Evolutionary conservation of site 1 and 2. Phylogenetic footprint analyses revealed a considerable conservation of the 2 kb *retSDR1* promoter region among several mammalian genomes. Particularly, we observed a strong evolutionary conservation of the identified p53 family-binding elements (data not shown).

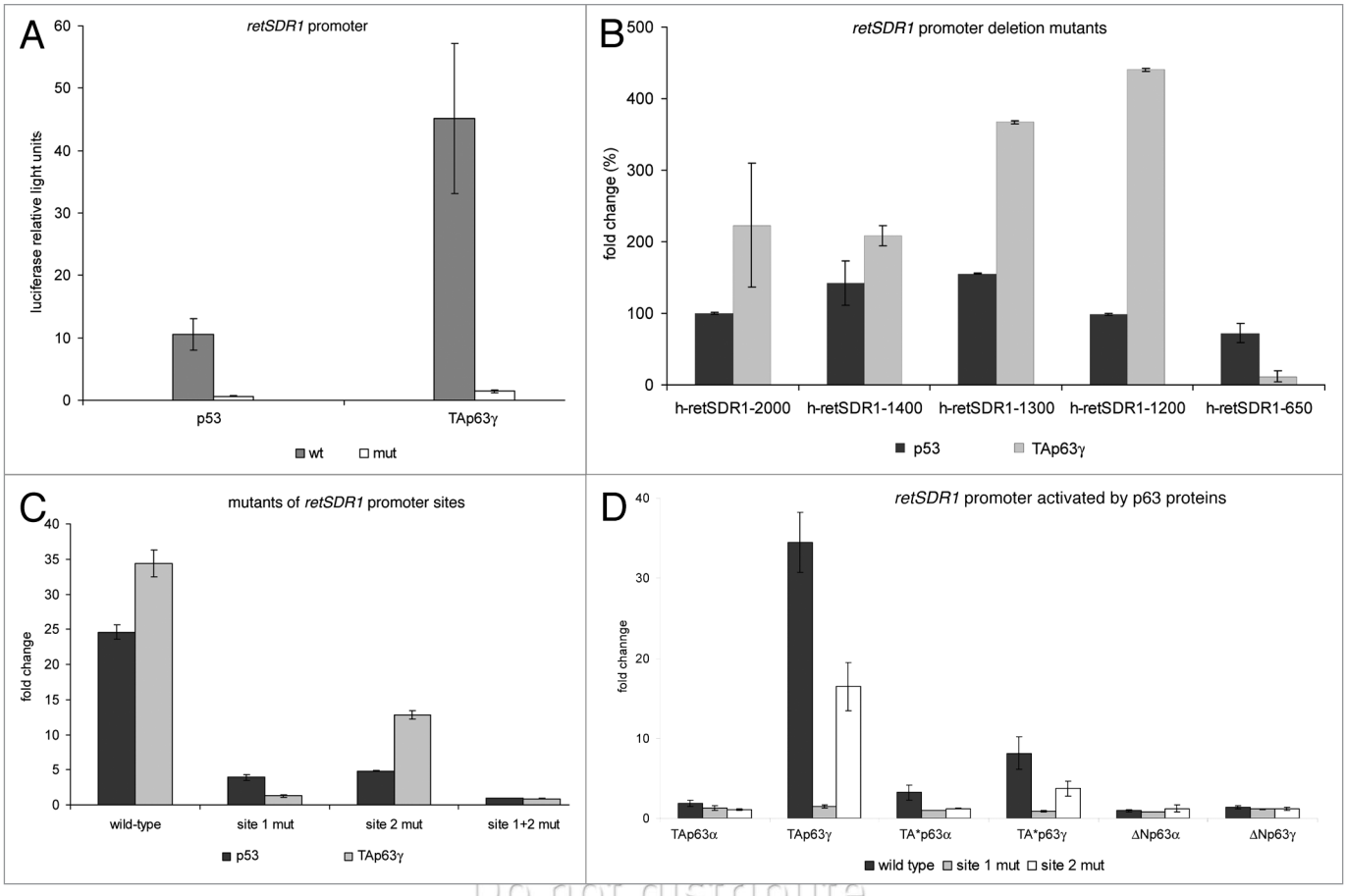

Figure 2. Transcription of *retSDR1* is regulated by p53 and p63 proteins. (A) The *retSDR1* promoter can be activated by p53 and TAp63γ. The h-retSDR1-2000 reporter was cotransfected with plasmids expressing with wild-type or mutant *p53* and *TAp63*γ. Transfections were performed in SaOS-2 cells using 250 ng of the *retSDR1* reporter plasmid with 25 ng of the plasmids expressing wild-type *TAp63*γ or *p53* (wt). Cotransfections of plasmids carrying DNA-binding deficient *p53* or *TAp63*γ mutants were utilized as negative controls (mut). All experiments were standardized to Renilla luciferase activity expressed from 25 ng pRL-null vector which was also cotransfected. Total amount of DNA transfected was held constant. Averages from three experiments with standard deviations are given. (B) Activation by either p53 or TAp63γ depends on different sections of the *retSDR1* promoter. Analyses of *retSDR1* promoter deletions following cotransfections of p53 and TAp63γ were performed as described above. Relative luciferase activities comparing measurements from activation by wild-type versus mutant p53 or TAp63γ are given as relative fold change. Activation by p53 with the full-length construct was set to 100%. (C) Identification of two elements responsible for transactivation by p53 or TAp63γ. Mutants on the basis of the h-retSDR1-1400 reporter construct of site 1 and site 2 were created separately or in combination. Luciferase reporter analyses were carried out as described above. Fold change induction is given comparing reporter activity after transfection of plasmids carrying wild-type or DNA-binding deficient *p53* as well as *TAp63*γ. (D) Various p63 proteins were tested for their activity to transactivate wild-type or mutant h-retSDR1-1400 reporter constructs. Luciferase reporter analyses were carried out as described above.

EEC syndrome-specific mutations of TAp63γ fail to transactivate *retSDR1*. Mutations of the DNA binding domain of p63 exhibit phenotypes that cause several autosomal dominantly inherited syndromes leading to developmental malformations. We tested the transcriptional regulation of the *retSDR1* promoter by mutant TAp63γ proteins originating from patients afflicted by such syndromes. The transcriptional effect of TAp63γ mutants derived from the EEC syndrome (R204W, R279H and R304A), the SHFM syndrome (K193E and K194E) and the ADULT syndrome (R298Q) were analyzed in reporter assays. The EEC-derived mutants lost the ability to transactivate *retSDR1*. Mutants of p63 found in SHFM patients yielded similar reporter activity as wild-type TAp63γ. A mutant related to the ADULT syndrome stimulated *retSDR1* transcription significantly less than the wild-type variant of p63 (**Fig. 5A**).

The DNA binding capability of the TAp63γ mutants was tested by EMSA. For these assays we employed the p53 consensus site of the $p21^{WAF1/CIP1}$ promoter as probe. Binding to the retSDR1 promoter can also be detected (**Fig. 3**). However, the lower signals obtained do not allow to discern subtle differences in p63 mutant binding. We observed that TAp63γ mutant proteins from EEC patients failed to bind to the p53 site, whereas mutants found in SHFM and ADULT syndromes were still able to bind the probe (**Fig. 5B**). This binding pattern of mutants is consistent with the ability to activate *retSDR1* in the reporter assay (**Fig. 5A**).

Correlation of p53 and p63γ protein binding to the *retSDR1* promoter with *retSDR1* expression following DNA damage. Different cell lines were treated with doxorubicin in order to analyze *retSDR1* regulation after DNA damage. Treatment with the chemotherapeutic results in p53 accumulation in p53-positive but

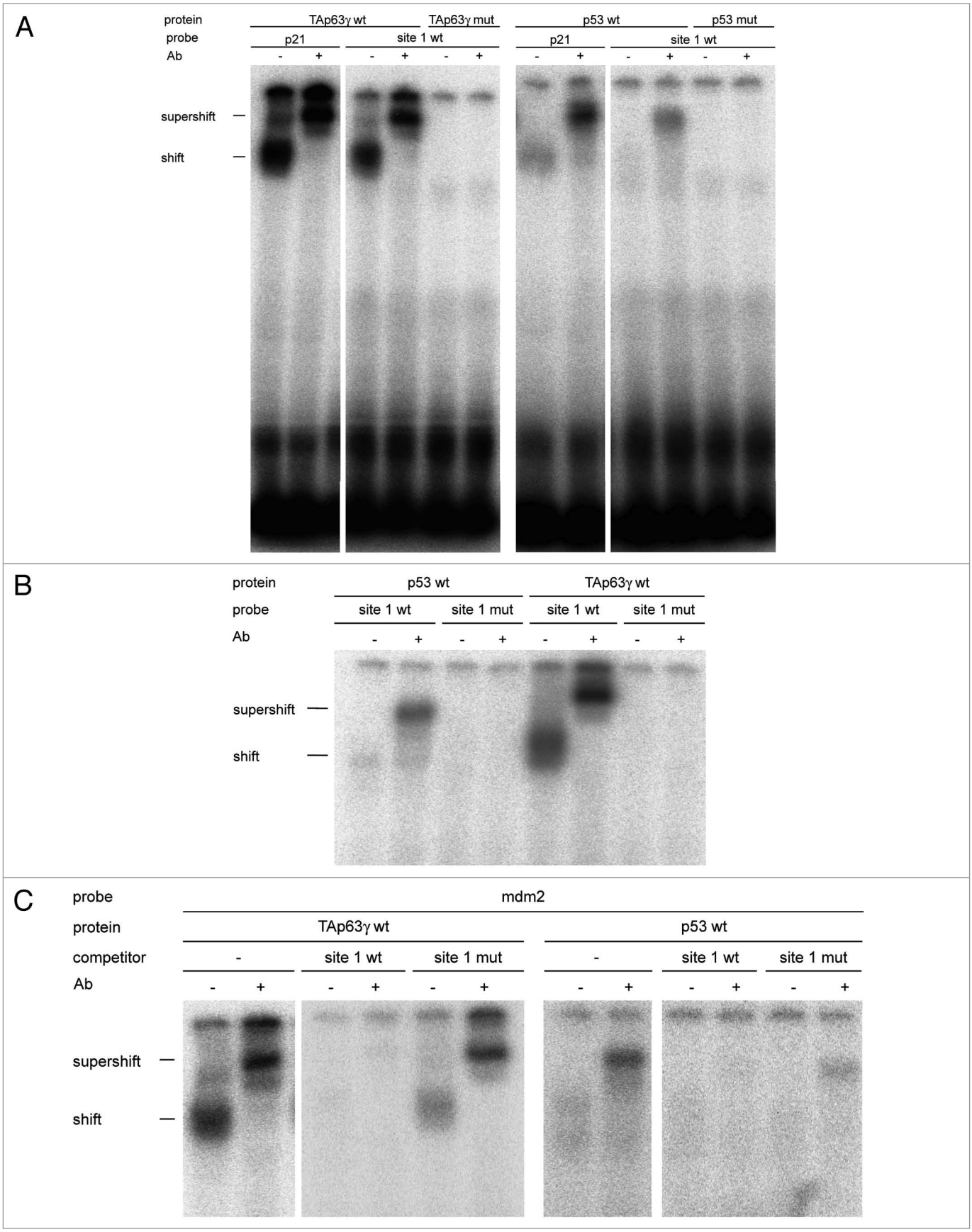

Figure 3. For figure legend, see page 2182.

Figure 3 (See previous page). TAp63γ and p53 bind to the novel site 1 in the *retSDR1* promoter in vitro. EMSAs testing site 1 from the *retSDR1* promoter for binding of TAp63γ and p53. (A) A p53 site from the *p21*$^{WAF1/CIP1}$ promoter served as positive binding control for p53 and TAp63γ translated in vitro. Mutant proteins deficient in DNA binding were employed as negative controls. Addition of antibody against p63γ or p53 yielded supershifted bands of specific complexes. (B) Binding of wild-type p53 or TAp63γ was assayed with wild-type site 1 (wt) oligonucleotide in comparison to a probe in which the binding element was destroyed (mut). (C) Unlabelled probes representing either wild-type or mutant site 1 from the *retSDR1* promoter were used to compete for wild-type TAp63γ and p53 protein binding to an oligonucleotide duplex carrying the p53 element from the *mdm2* promoter. Unlabelled probe was applied with a 100-fold excess over radioactive oligonucleotides.

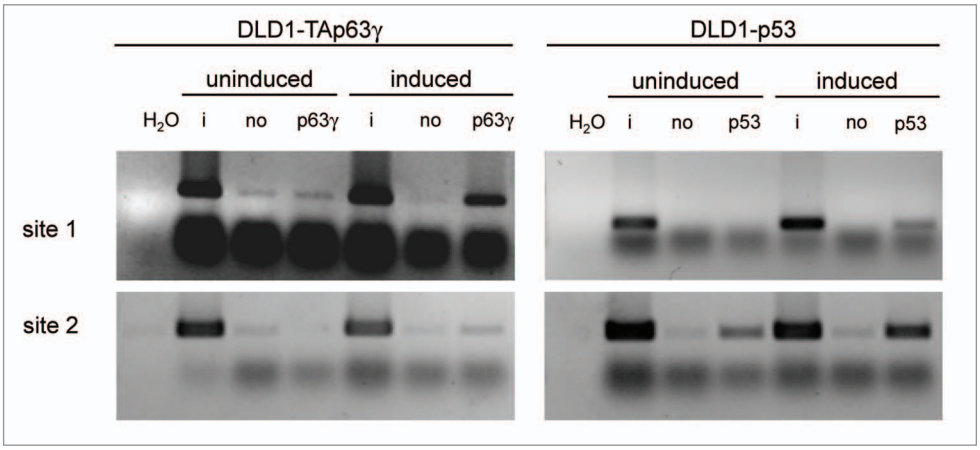

Figure 4. In vivo binding of TAp63γ and p53 proteins to the *retSDR1* promoter. ChIP assays were performed with the *retSDR1* promoter. Chromatin from DLD-1 cells expressing p53 or TAp63γ proteins via a tet-off system was crosslinked before (uninduced) and after induction (induced). After DNA extraction and precipitation with antibodies against p63γ or p53, fragments were amplified by PCR with primers for *retSDR1* promoter regions containing site 1 or site 2. Amplified fragments were separated on a gel. Input (i); no antibody (no); water control (H_2O).

not in p53-negative HCT116 cells.[40] Consistent with the results described above, we observed a strong upregulation of *retSDR1* mRNA following p53 protein expression when HCT116 *p53*$^{+/+}$ cells were treated with doxorubicin (**Fig. 6A**). Importantly, this correlates with the recruitment of p53 in vivo to the *retSDR1* promoter in p53-positive but not in p53-negative HCT116 cells (**Fig. 6B**).

It has been observed earlier that p63 expression could be induced by DNA damage.[42] However, expression levels of p63 in the HCT116 cell system are not sufficient to analyze the recruitment of p63 to the *retSDR1* promoter by ChIP (**Fig. 6B** and data not shown). Therefore, we tested p63-expressing HepG2 and SaOS-2 cells after doxorubicin treatment (**Fig. 6B**). Similar to the findings in p53-positive HCT116 cells, increased expression of *retSDR1* mRNA was measured following DNA damage also in HepG2 and SaOS-2 cells (**Fig. 6A**). This correlates with the recruitment of p53 as well as p63γ protein in vivo to the *retSDR1* promoter in HepG2 cells after doxorubicin treatment. In p53-negative SaOS-2 cells p63γ protein appears to bind to the *retSDR1* promoter, while no accumulation of p53 protein is detectable (**Fig. 6B**). The induction of *retSDR1* expression after DNA damage in p53-negative SaOS-2 cells and the increased binding of p63 protein to the promoter argue in favor of a function for p63γ proteins in regulating *retSDR1* also in vivo.

Expression of *retSDR1* correlates with *TAp63* expression in human colon carcinomas. Retinoids are not only involved in differentiation but also in proliferation. Emphasizing a possible role of retSDR1 in these processes it had been shown that *retSDR1* is expressed in most tissues and its overexpression contributes to retinyl ester storage in cells.[9] Consistent with a function in cancer development, it was shown that *retSDR1* is located on a chromosomal fragment often deleted in neuroblastoma.[9] In order to further obtain information on a possible function of retSDR1 in tumorigenesis, expression of its mRNA in human colorectal carcinomas was analyzed. However, a few caveats have to be considered for this experiment. Since the samples did not consist of pure tumor cells, some of the non-tumor cells may reflect a wild-type situation. Furthermore, it cannot be excluded that p53, which is likely inactivated in colorectal adenocarcinomas, or other transcription factors influence expression of p53 targets differentially in the tumor and non-tumor tissues. Nevertheless, we observed a significant increase of *retSDR1* mRNA in tumor samples compared to healthy tissues from the same individuals (**Fig. 6C**). This expression pattern correlates well with the expression of TAp63 isoforms. Furthermore, expression of *p21*$^{WAF1/CIP1}$, as a classical target gene of the p53/p63 signaling pathway, was also significantly increased in tumor tissues. Taken together, these results may imply that TAp63 proteins induce expression of *retSDR1* in carcinomas to counteract tumor progression by supporting retinoid function.

Discussion

Fundamental biological processes such as embryonic development, vision, fertility, immune response, cellular differentiation and maintenance of various epithelia require vitamin A and its metabolites. Another important function of an efficient vitamin A metabolism is observed in tumor prevention.[11]

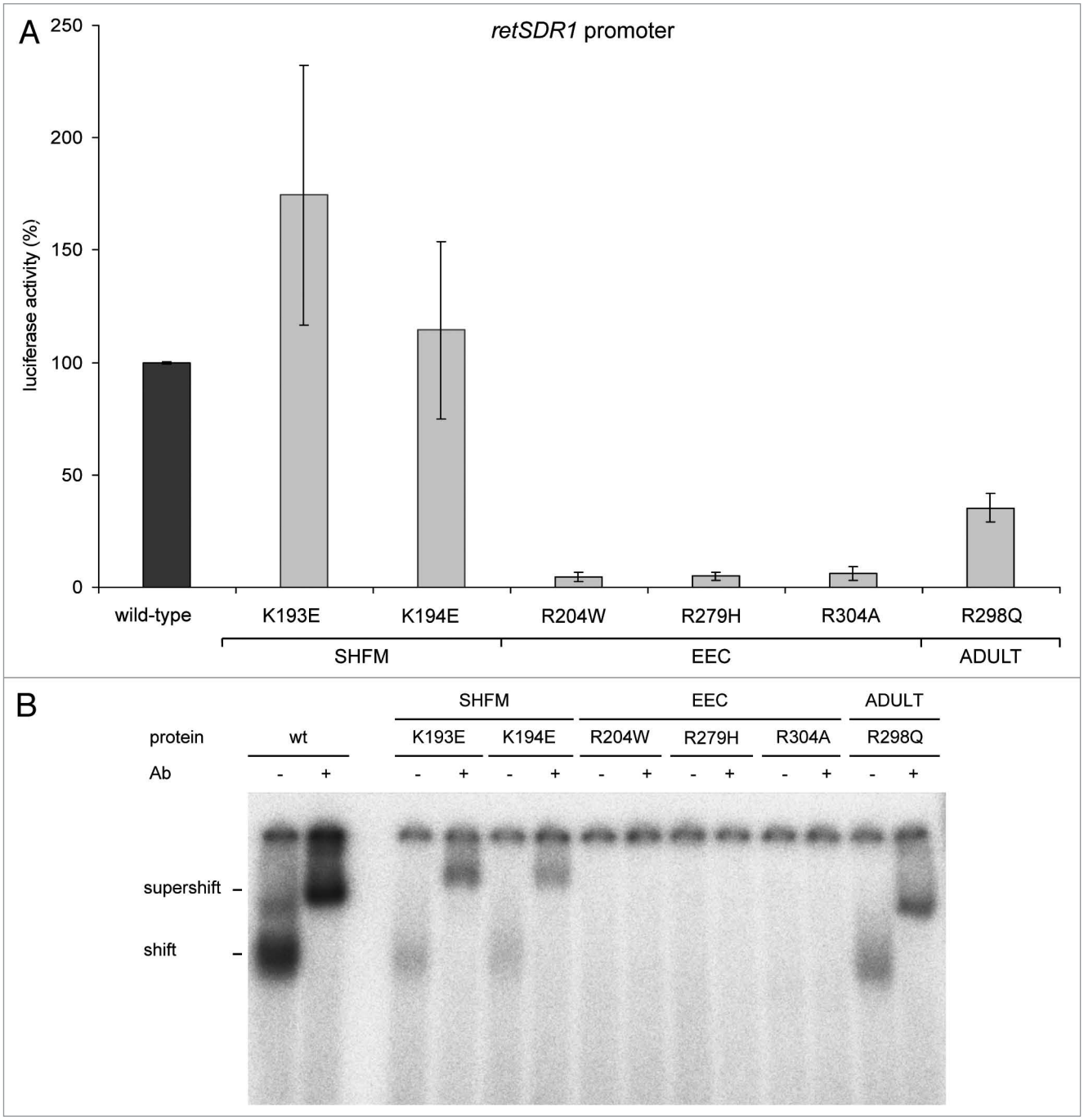

Figure 5. Mutants of TAp63γ related to human developmental syndromes show a significant functional difference in activating *retSDR1* transcription. (A) TAp63γ mutants related to the human developmental syndromes SHFM, EEC and ADULT mediate differentially activation of the h-retSDR1-1400 reporter construct. Luciferase reporter assays were performed as described in **Figure 2**. Relative light units are given in percent relative to reporter activity of wild-type TAp63γ which was set to 100%. (B) DNA binding of TAp63γ mutants was analyzed by EMSA as described in Figure 3. In vitro translated mutants of TAp63γ were incubated with a probe containing the p53 consensus site of the *p21* promoter. Specificity of the detected interaction was verified by adding an antibody against p63γ resulting in a supershift.

Here we describe the *retSDR1* gene, which codes for an enzyme central to the vitamin A metabolism, as a transcriptional target of the p53 family of proteins. Particularly, TAp63γ, an important representative of the p63 subfamily and structurally most closely related to p53, strongly activates *retSDR1* transcription. Consistent with our observation, the induction of *retSDR1* expression was also detected in a microarray-based screening when TAp63γ was overexpressed in a different cell system.[43]

The retSDR1 enzyme appears to fulfill a much larger number of functions than being part of the visual cycle regenerating retinoids for which it was originally identified.[7] Expression of *retSDR1* on the mRNA level was observed in nearly all tissues

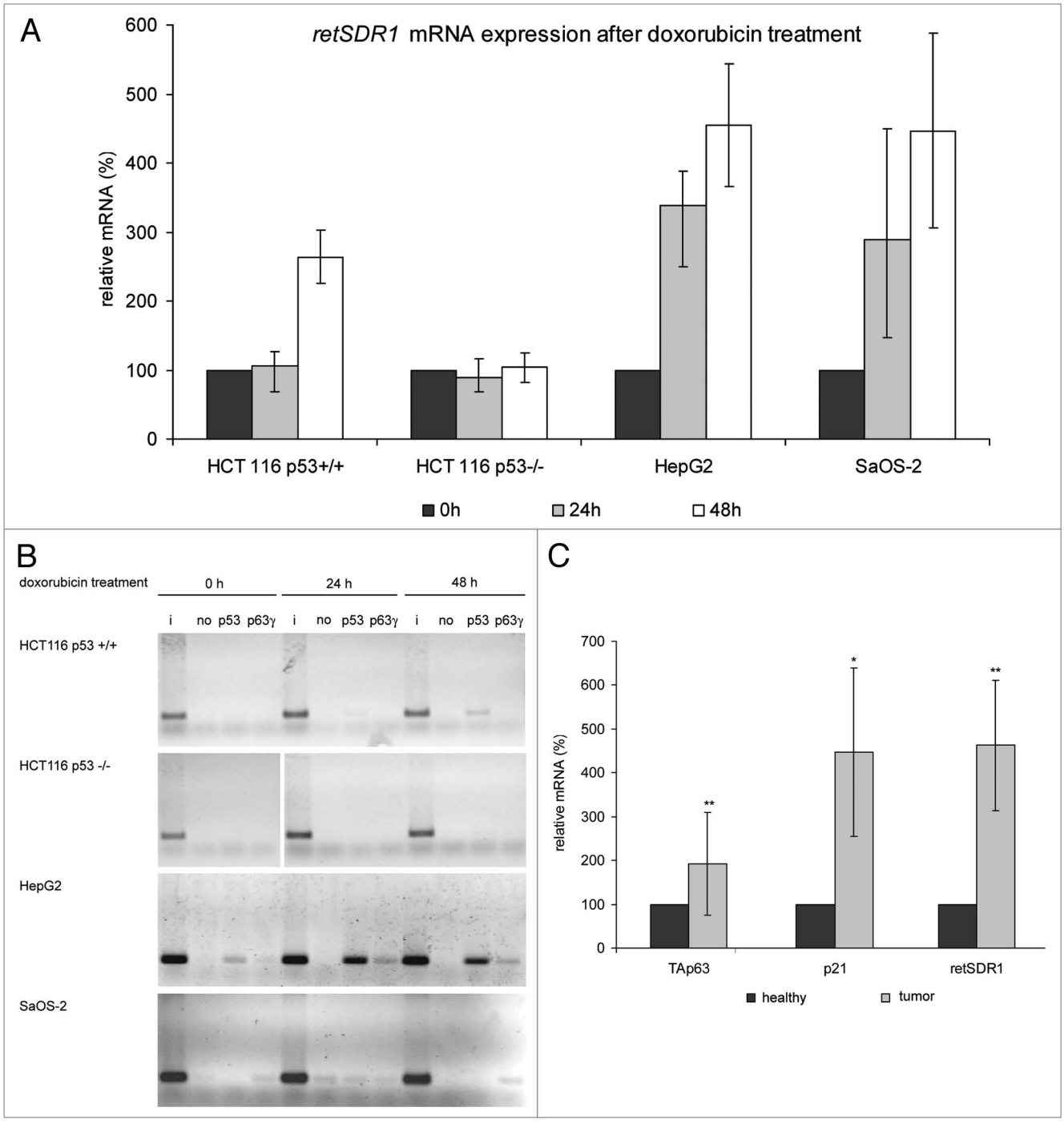

Figure 6. Expression of *retSDR1* is activated by p53/p63 following DNA damage and correlates with expression of *TAp63* proteins in human colon carcinoma cells. (A) Following DNA damage due to doxorubicin treatment for indicated time points *retSDR1* mRNA expression was measured by semiquantitative real-time RT-PCR. HCT116 cells expressing wild-type p53, p53-negative HCT116 cells, p53- and TAp63-expressing HepG2 cells, and p53-negative SaOS-2 cells which express TAp63 proteins were analyzed. Expression of *retSDR1* in untreated cells (0 h) served as control and was set to 100%. *GAPDH* expression was used for standardization. (B) Differential recruitment of p53 and p63γ proteins to the *retSDR1* promoter following doxorubicin treatment was analyzed by chromatin immunoprecipitation (ChIP) as described above; i, input; no, no antibody; p53, IP with p53-specific antibody; p63γ IP with p63γ-specific antibody. (C) The mRNA expression of *retSDR1*, *TAp63 iso*forms and *p21*$^{WAF1/CIP1}$ in human colon carcinomas was determined by real-time RT-PCR and normalized to *GAPDH* mRNA expression. Relative mRNA amounts from four patients are given in percent. As control mRNA expression from healthy tissue close to the tumor site was measured and set to 100%. Significance was determined by Student's t-test. (**$p \leq 0.01$; *$p \leq 0.05$; $n = 4$).

that were tested. Particularly high expression levels were found in fetal kidney, liver, and lung and in adult heart, placenta, lung, liver, kidney, pancreas, thyroid, testis, stomach, trachea and spinal cord.[9] It is not obvious what the retSDR1 functions in these tissues are. Developmental defects resulting from a lack of retinoids have been observed in most of the tissues in which retSDR1 is expressed, e.g., rat embryos developing in mothers maintained before and during pregnancy on a vitamin A-deficient diet are affected by malformations particularly in eyes, kidneys, ureters and genital ducts.[1] The wide tissue distribution of processes dependent on vitamin A has also been demonstrated by STRA6. This membrane protein binds the retinol-binding protein RBP and thereby allows transport of vitamin A from RBP-vitamin A complexes across outer cell membranes. STRA6 is widely expressed in different embryonal and adult tissues.[44] Consistent with a broad expression pattern and its central function in vitamin A resorption, mutation of *STRA6* leads to malformations in humans which are similar to developmental malformations seen in animal embryos developing under vitamin A deficiency.[1,45] Such examples confirm a broad spectrum of functions for retinoid metabolism-linked proteins. Therefore, retSDR1 expression observed in other tissues than the retina, especially in embryos and differential expression in a cancer context, indicates wider retSDR1 functions particularly in development and proliferation.

Several reports indicate a role for retSDR1 in tumor development. Elevated *retSDR1* mRNA expression was detected in papillary thyroid carcinoma compared to normal adjacent thyroid tissue.[46] Expression of *retSDR1* transcripts was not observed in follicular thyroid carcinoma. The two tumor types differ clinically. Therefore, *retSDR1* expression may serve as marker hinting to biological differences between both tumor forms. Furthermore, *retSDR1* was found prominently expressed in nonmetastatic relative to metastatic papillary thyroid carcinoma.[46] Another recent study found *retSDR1* expression altered in radiation-induced papillary thyroid carcinoma.[47] Consistent with these observations, CpG-island methylation of the *retSDR1* promoter was detected in human malignant melanomas likely resulting in gene silencing in cancer cells.[10]

Another cancer-related aspect is that *retSDR1* expression was shown to be induced by bexarotene. This member of the rexinoid class of retinoid X receptor-binding ligands prevents development of premalignant mammary lesions. The induction of *retSDR1* was implicated to function in the chemoprevention of mammary tumors by bexarotene.[48] In a recent clinical study, a correlation between elevated *retSDR1* expression and favorable outcome of childhood neuroblastoma was discovered. Even after chemotherapy, patients with increased expression fared better than cases with low *retSDR1* mRNA levels.[49]

Generally, it is well accepted that vitamin A deficiency leads to increased development of spontaneous and chemically induced tumors.[11] Furthermore, expression of *retSDR1* in neuroblastoma cells induces accumulation of retinyl ester as a storage form of vitamin A.[9] This report clearly associates expression of retSDR1 in a tissue not related to the visual cycle with producing storage forms of vitamin A which then are available for vitamin A functions in all tissues in which retSDR1 is expressed. The general supply with retinol derivatives, like retinoic acid, likely serves to inhibit proliferation,[12-14,50] to initiate differentiation[15] or to mediate apoptosis.[16,17]

We detected clearly elevated levels of *retSDR1* mRNA in human colon carcinomas compared to normal tissue. Expression levels of *retSDR1* correlated well with expression of TAp63 isoforms. However, the tumor samples did not exclusively consist of tumor cells and therefore the p53 status had not been analyzed. Thus, it cannot be excluded that $p21^{WAF1/CIP1}$ and *retSDR1* expression is influenced by p53 or other transcription factors. Nevertheless, the observed correlation in expression may suggest a compensatory mechanism through p53/p63-mediated regulation of *retSDR1* to inhibit tumor progression. Supporting this idea, we found a pronounced induction of *retSDR1* mRNA following DNA damage by doxorubicin treatment in several cell lines. Increased *retSDR1* transcription correlated well with p53 accumulation and the increased expression of TAp63 isoforms (data not shown). Additionally, recruitment of p53 as well as p63γ protein to the *retSDR1* promoter was detected in vivo after DNA damage.

Generally, a function for TAp63 in cellular senescence induction and tumor suppression independent of p53 has been established.[51] Therefore, expression of TAp63 isoforms in tumor tissues and recruitment of p63γ proteins to the *retSDR1* promoter together with a favorable role of p63 in tumor suppression may indicate that some of these p63 functions are controlled through retinoid synthesis. Taken together, this provides evidence for a role of *retSDR1* transcriptional regulation by p53/p63-family members in tumor inhibition.

It is well established that retinoic acid as a vitamin A-derived morphogen has a pivotal role during development. Vitamin A metabolism is essential during embryogenesis, since its dysregulation leads to severe malformations mainly due to a false pattern formation of the embryonal anterioposterior body axis.[1,52] Many of the developmental defects could be linked on a molecular level to retinoic acid metabolism.[2] Also for retSDR1, as an enzyme involved in retinol metabolism, several results are published that hint to a function of retSDR1 in differentiation.[9] One such example was reported from the differentiation of buccal keratinocytes. When normal keratinocytes are cultivated in serum concentrations inducing terminal differentiation of squamous cells, differentiated cells show elevated *retSDR1* expression compared to cells before serum stimulation.[53]

Also p63 proteins when mutated were found responsible for a family of human malformation syndromes. Several different but related malformation patterns constitute the EEC, AEC, SHFM, LMS and ADULT syndromes which are characterized by combinations of ectrodactyly, ectodermal dysplasia and facial clefting.[36,37,54] p63 mutations occurring in EEC syndrome such as R204, R279 and R304 correspond to hotspot mutations in the DNA binding domain of p53 destroying protein/DNA interaction.[38] Mutations responsible for the SHFM-syndrome phenotype, such as K193 or K194, do not primarily impair DNA binding. Interesting to note is that in contrast to people affected by the EEC or ADULT syndromes, SHFM patients lack epidermal dysplasia.[55]

Here, we observed a differential regulation of *retSDR1* expression by TAp63γ mutants derived from a group of human malformation syndromes. These mutations are known to differentially affect transactivation of p53/p63-responsive genes.[56] TAp63γ mutants derived from EEC patients were not able to bind to a p53 consensus element and consequently did not significantly transactivate *retSDR1*. However, mutants responsible for the SHFM phenotype, such as K193 or K194, as well as the mutant protein derived from ADULT-syndrome patients bound to the p53 site. These mutant proteins could still activate *retSDR1* transcription, but the mutant from ADULT-syndrome patients activated *retSDR1* to a lesser extend when compared to wild-type TAp63γ. Importantly, phenotypic variation of EEC, ADULT and SHFM syndromes corresponded well with the activation of the *retSDR1* promoter caused by specific p63 mutants related to the developmental defects.[36,55,57] Considering these observations together with the role of retSDR1 in retinol metabolism, one may speculate that the ability of p63 to stimulate *retSDR1* expression contributes to the phenotype development in these patients.

Materials and Methods

Cell culture, transfections and luciferase assays. SaOS-2 cells obtained from DSMZ (Braunschweig, Germany) were cultured and transfected as described previously.[39,40] Transient transfections were carried out according to the manufacturer's instructions using Fugene 6 (Roche, Mannheim, Germany). Expression plasmids for wild-type or mutant *p53/p63* family members (25 ng) with 400 ng of the plasmid carrying the human *retSDR1* promoter and 25 ng of the pRL-null vector (Promega, Mannheim, Germany) were transfected per assay. The total amount of transfected DNA was held constant. Luciferase assays were carried out as reported earlier.[40]

Parental HCT116 cells and HCT116 cells with targeted deletion in both p53 alleles as well as an inducible DLD-1-tet-off-p53 cell line D.P53 A2 were generously provided by Bert Vogelstein and cultured as described.[40] HepG2 cells were obtained from DSMZ (Braunschweig, Germany) and cultured as described.[40] DNA damaging was carried out by treating the cells with 0.2 μg/ml doxorubicin.

RNA extraction and real-time RT-PCR. Extraction of total RNA was performed using the RNeasy Mini Kit (Qiagen, Hilden, Germany). DLD-1 colon carcinoma cells carrying *p53*-family transgenes used for RNA preparations were described earlier.[39,58] RNA extraction from tissue samples was done using the Trizol reagent (Invitrogen). Real-time RT-PCR mRNA quantification including calculations have been described.[40] Specific primers for human *retSDR1,* 5'-CAA GTC CCA ACA CAT CAA CAC C-3'; 5'-CTC TCC ATG AAG GCG AAG G-3'; *p53,* 5'-ATG GAG GAG CCG CAG TCA GAT C-3'; 5'-CCA TTG TTC AAT ATC GTC CGG G-3'; *TAp63* isoforms, 5'-ATG TCC CAG AGC ACA CAG AC-3'; 5'-CAC ATG GGG TCA CTC AGG TC-3'; *p21,* 5'-GTC AGA ACC CAT GCG GCA GCA AG-3'; 5' CAG GTC CAC ATG GTC TTC CTC TG 3' were used at 1 μM on 50 ng total RNA template in the QuantiTect SYBR Green RT-PCR Mix (Qiagen) in the LightCycler instrument (Roche).[59]

Western analysis. Western blots were prepared essentially as previously described.[40] For detection of retSDR1 the monoclonal mouse anti-human retSDR1 antibody mAB A_{11} was used in a 1:1,000 dilution.[7] We are very grateful to Francoise Haeseleer for generously providing this antibody preparation. The blot was stripped and reprobed with a 1:5,000 dilution of the mouse monoclonal anti-β-actin antibody (clone AC-15, Sigma, Taufkirchen, Germany). The induction of p53 was detected with the mouse monoclonal antibody DO-1 (Calbiochem, Darmstadt, Germany; 1:1,000 dilution). The induction of TAp63γ was detected using the mouse monoclonal anti-p63 antibody (clone 4A4; sc-8431 Santa Cruz Biotechnology, Santa Cruz, CA, USA) with a 1:1,000 dilution.

Cloning and mutation of the human *retSDR1* promoter constructs and *p63* expression plasmids. Human genomic DNA prepared from blood was used to amplify a fragment of the *retSDR1* promoter 1,966 bp upstream of the translation start including 43 bp of coding sequence with the primers 5'-GAA GAT CTA ATC GCC GTC TGA ACA AGT C-3' and 5'-CGT AGC CAT GGC TAG AGG GAA CAT CAC CAG CG-3' (GenBank accession number EU178141). This fragment was ligated into the *Bgl*II/*Nco*I sites of the pGL3 basic vector (Promega, Mannheim, Germany) yielding h-retSDR1-2000. The promoter sequence was submitted to the data base (GenBank accession number EU178141). Promoter deletions and targeted mutations of site 1 and 2 were created by PCR and confirmed by sequencing. TAp63γ mutants were created by targeted mutagenesis as described previously.[40] TAp63γ-R304H was described earlier.[39]

Electrophoretic mobility shift assays (EMSA). EMSAs were carried out as described previously.[40,60] For supershifts goat polyclonal anti-p63γ antibody (C-18, sc-8370, Santa Cruz Biotechnology, Santa Cruz, CA, USA) or monoclonal mouse anti-p53 antibody (pAb421, SA-293, Biomol, Hamburg, Germany) were employed. Double-stranded probes were generated, just forward oligonucleotides are given: hretSDR1-site1, 5'-GGA CAC CGG CGG CAA GTC CCG GCT TGC AGA GAG GG-3'; hretSDR1-site1-mut, 5'-GGA CAC CGG CGG TCG TGC CCG GTC GTC AGA GAG GG-3'; h-retSDR1-site2-wt, 5'-GGG CTG GGG CAA GAA ACT TCT TGT TAG AAC TTT CC-3'; hretSDR1-site2-mut, 5'-GGG CTG GGG TGA TAA ACT TTG TTT TAG AAC TTT CC-3'; p21, 5'-GGC CAT CAG GAA CAT GTC CCA ACA TGT TGA GCT CT-3'; mdm2, 5'-GGG CGG CCG CTG GTC AAG TTG GGA CAC GTC CGG-3'.

Chromatin immunoprecipitation (ChIP) assays. ChIPs were performed as published.[61] Protein crosslinks were precipitated using 5 μg of a goat polyclonal anti-p63γ antibody (C-18, sc-8370, Santa Cruz Biotechnology) or monoclonal anti-p53 (DO-1, Calbiochem, Darmstadt, Germany) and analyzed as described earlier.[40] Employing the primers hretSDR1-chip-site1-fwd, 5'-GCA AGG AGG AAC GTC TCG-3'; hretSDR1-chip-site1-rev, 5'-GTT CCT CTG AGT GGC TGC-3'; hretSDR1-chip-site2-fwd, 5'-GGG CGC TAC AAT TTG GAA

TC-3'; hretSDR1-chip-site2-rev, 5'-CAC CTG GCC ACT CTT GAA ATC-3' products were amplified.

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Note

Supplementary materials can be found at:
www.landesbioscience.com/supplement/KirschnerCC9-11-Sup.pdf

References

1. Wilson JG, Roth CB, Warkany J. An analysis of the syndrome of malformations induced by maternal vitamin A deficiency. Effects of restoration of vitamin A at various times during gestation. Am J Anat 1953; 92:189-217.
2. Niederreither K, Dolle P. Retinoic acid in development: towards an integrated view. Nat Rev Genet 2008; 9:541-53.
3. Mark M, Ghyselinck NB, Chambon P. Function of retinoic acid receptors during embryonic development. Nucl Recept Signal 2009; 7:002.
4. Blomhoff R, Blomhoff HK. Overview of retinoid metabolism and function. J Neurobiol 2006; 66:606-30.
5. Pares X, Farres J, Kedishvili N, Duester G. Medium- and short-chain dehydrogenase/reductase gene and protein families : Medium-chain and short-chain dehydrogenases/reductases in retinoid metabolism. Cell Mol Life Sci 2008; 65:3936-49.
6. Duester G. Retinoic acid synthesis and signaling during early organogenesis. Cell 2008; 134:921-31.
7. Haeseleer F, Huang J, Lebioda L, Saari JC, Palczewski K. Molecular characterization of a novel short-chain dehydrogenase/reductase that reduces all-trans-retinal. J Biol Chem 1998; 273:21790-9.
8. Bray JE, Marsden BD, Oppermann U. The human short-chain dehydrogenase/reductase (SDR) superfamily: a bioinformatics summary. Chem Biol Interact 2009; 178:99-109.
9. Cerignoli F, Guo X, Cardinali B, Rinaldi C, Casaletto J, Frati L, et al. retSDR1, a short-chain retinol dehydrogenase/reductase, is retinoic acid-inducible and frequently deleted in human neuroblastoma cell lines. Cancer Res 2002; 62:1196-204.
10. Furuta J, Nobeyama Y, Umebayashi Y, Otsuka F, Kikuchi K, Ushijima T. Silencing of Peroxiredoxin 2 and aberrant methylation of 33 CpG islands in putative promoter regions in human malignant melanomas. Cancer Res 2006; 66:6080-6.
11. Niles RM. Signaling pathways in retinoid chemoprevention and treatment of cancer. Mutat Res 2004; 555:81-96.
12. Langenfeld J, Kiyokawa H, Sekula D, Boyle J, Dmitrovsky E. Posttranslational regulation of cyclin D1 by retinoic acid: a chemoprevention mechanism. Proc Natl Acad Sci USA 1997; 94:12070-4.
13. Matsuo T, Thiele CJ. $p27^{Kip1}$: a key mediator of retinoic acid induced growth arrest in the SMS-KCNR human neuroblastoma cell line. Oncogene 1998; 16:3337-43.
14. Teixeira C, Pratt MA. CDK2 is a target for retinoic acid-mediated growth inhibition in MCF-7 human breast cancer cells. Mol Endocrinol 1997; 11:1191-202.
15. Chen LC, Sly L, De Luca LM. High dietary retinoic acid prevents malignant conversion of skin papillomas induced by a two-stage carcinogenesis protocol in female SENCAR mice. Carcinogenesis 1994; 15:2383-6.
16. Fujimura S, Suzumiya J, Anzai K, Ohkubo K, Hata T, Yamada Y, et al. Retinoic acids induce growth inhibition and apoptosis in adult T-cell leukemia (ATL) cell lines. Leuk Res 1998; 22:611-8.
17. Hsu SL, Yin SC, Liu MC, Reichert U, Ho WL. Involvement of cyclin-dependent kinase activities in CD437-induced apoptosis. Exp Cell Res 1999; 252:332-41.
18. Murray-Zmijewski F, Lane DP, Bourdon JC. p53/p63/p73 isoforms: an orchestra of isoforms to harmonise cell differentiation and response to stress. Cell Death Differ 2006; 13:962-72.
19. Blandino G, Dobbelstein M. p73 and p63: why do we still need them? Cell Cycle 2004; 3:886-94.
20. Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CA Jr, Butel JS, Bradley A. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature 1992; 356:215-21.
21. Harvey M, McArthur MJ, Montgomery CA Jr, Butel JS, Bradley A, Donehower LA. Spontaneous and carcinogen-induced tumorigenesis in p53-deficient mice. Nat Genet 1993; 5:225-9.
22. Choi J, Donehower LA. p53 in embryonic development: maintaining a fine balance. Cell Mol Life Sci 1999; 55:38-47.
23. Sasai N, Yakura R, Kamiya D, Nakazawa Y, Sasai Y. Ectodermal factor restricts mesoderm differentiation by inhibiting p53. Cell 2008; 133:878-90.
24. Vousden KH, Lane DP. p53 in health and disease. Nat Rev Mol Cell Biol 2007; 8:275-83.
25. Böhlig L, Metzger R, Rother K, Till H, Engeland K. The CCN3 gene coding for an extracellular adhesion-related protein is transcriptionally activated by the p53 tumor suppressor. Cell Cycle 2008; 7:1254-61.
26. Buganim Y, Rotter V. RHAMM in the complex p53 cell cycle network. Cell Cycle 2008; 7:3291.
27. Godar S, Weinberg RA. Filling the mosaic of p53 actions: p53 represses RHAMM expression. Cell Cycle 2008; 7:3479.
28. Sohr S, Engeland K. RHAMM is differentially expressed in the cell cycle and downregulated by the tumor suppressor p53. Cell Cycle 2008; 7:3448-60.
29. Kay C, Jeyendran RS, Coulam CB. p53 tumour suppressor gene polymorphism is associated with recurrent implantation failure. Reprod Biomed Online 2006; 13:492-6.
30. Hu W, Feng Z, Teresky AK, Levine AJ. p53 regulates maternal reproduction through LIF. Nature 2007; 450:721-4.
31. Mills AA, Zheng B, Wang XJ, Vogel H, Roop DR, Bradley A. p63 is a p53 homologue required for limb and epidermal morphogenesis. Nature 1999; 398:708-13.
32. Yang A, Schweitzer R, Sun D, Kaghad M, Walker N, Bronson RT, et al. p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. Nature 1999; 398:714-8.
33. Yang A, McKeon F. p63 and p73: p53 mimics, menaces and more. Nat Rev Mol Cell Biol 2000; 1:199-207.
34. Vigano MA, Lamartine J, Testoni B, Merico D, Alotto D, Castagnoli C, et al. New p63 targets in keratinocytes identified by a genome-wide approach. EMBO J 2006; 25:5105-16.
35. Brunner HG, Hamel BC, van Bokhoven H. The p63 gene in EEC and other syndromes. J Med Genet 2002; 39:377-81.
36. Rinne T, Brunner HG, van Bokhoven H. p63-associated disorders. Cell Cycle 2007; 6:262-8.
37. van Bokhoven H, Hamel BC, Bamshad M, Sangiorgi E, Gurrieri F, Duijf PH, et al. p63 Gene mutations in eec syndrome, limb-mammary syndrome, and isolated split hand-split foot malformation suggest a genotype-phenotype correlation. Am J Hum Genet 2001; 69:481-92.
38. van Bokhoven H, Brunner HG. Splitting p63. Am J Hum Genet 2002; 71:1-13.
39. Dietz S, Rother K, Bamberger C, Schmale H, Mössner J, Engeland K. Differential regulation of transcription and induction of programmed cell death by human p53-family members p63 and p73. FEBS Lett 2002; 525:93-9.
40. Kirschner RD, Sänger K, Müller GA, Engeland K. Transcriptional activation of the tumor suppressor and differentiation gene S100A2 by a novel p63-binding site. Nucleic Acids Res 2008; 36:2969-80.
41. Deyoung MP, Ellisen LW. p63 and p73 in human cancer: defining the network. Oncogene 2007; 26:5169-83.
42. Petitjean A, Cavard C, Shi H, Tribollet V, Hainaut P, Caron dF. The expression of TA and DeltaNp63 are regulated by different mechanisms in liver cells. Oncogene 2005; 24:512-9.
43. Yang A, Zhu Z, Kapranov P, McKeon F, Church GM, Gingeras TR, Struhl K. Relationships between p63 binding, DNA sequence, transcription activity and biological function in human cells. Molecular Cell 2006; 24:593-602.
44. Kawaguchi R, Yu J, Honda J, Hu J, Whitelegge J, Ping P, et al. A membrane receptor for retinol binding protein mediates cellular uptake of vitamin A. Science 2007; 315:820-5.
45. Pasutto F, Sticht H, Hammersen G, Gillessen-Kaesbach G, Fitzpatrick DR, Nurnberg G, et al. Mutations in STRA6 cause a broad spectrum of malformations including anophthalmia, congenital heart defects, diaphragmatic hernia, alveolar capillary dysplasia, lung hypoplasia and mental retardation. Am J Hum Genet 2007; 80:550-60.
46. Oler G, Camacho CP, Hojaij FC, Michaluart P Jr, Riggins GJ, Cerutti JM. Gene expression profiling of papillary thyroid carcinoma identifies transcripts correlated with BRAF mutational status and lymph node metastasis. Clin Cancer Res 2008; 14:4735-42.
47. Stein L, Rothschild J, Luce J, Cowell JK, Thomas G, Bogdanova TI, et al. Copy Number and Gene Expression Alterations in Radiation-Induced Papillary Thyroid Carcinoma from Chernobyl Pediatric Patients. Thyroid 2009; In press.
48. Li Y, Zhang Y, Hill J, Kim HT, Shen Q, Bissonnette RP, et al. The rexinoid, bexarotene, prevents the development of premalignant lesions in MMTV-erbB2 mice. Br J Cancer 2008; 98:1380-8.
49. Kamei N, Hiyama K, Yamaoka H, Kamimatsuse A, Onitake Y, Sueda T, Hiyama E. Evaluation of genes identified by microarray analysis in favorable neuroblastoma. Pediatr Surg Int 2009; 25:931-7.
50. Langenfeld J, Lonardo F, Kiyokawa H, Passalaris T, Ahn MJ, Rusch V, Dmitrovsky E. Inhibited transformation of immortalized human bronchial epithelial cells by retinoic acid is linked to cyclin E downregulation. Oncogene 1996; 13:1983-90.

51. Guo X, Keyes WM, Papazoglu C, Zuber J, Li W, Lowe SW, et al. TAp63 induces senescence and suppresses tumorigenesis in vivo. Nat Cell Biol 2009; 11:1451-7.
52. Marletaz F, Holland LZ, Laudet V, Schubert M. Retinoic acid signaling and the evolution of chordates. Int J Biol Sci 2006; 2:38-47.
53. Staab CA, Ceder R, Roberg K, Grafstrom RC, Hoog JO. Serum-responsive expression of carbonyl-metabolizing enzymes in normal and transformed human buccal keratinocytes. Cell Mol Life Sci 2008; 65:3653-63.
54. van Bokhoven H, McKeon F. Mutations in the p53 homolog p63: allele-specific developmental syndromes in humans. Trends Mol Med 2002; 8:133-9.
55. Ianakiev P, Kilpatrick MW, Toudjarska I, Basel D, Beighton P, Tsipouras P. Split-hand/split-foot malformation is caused by mutations in the p63 gene on 3q27. Am J Human Genet 2000; 67:59-66.
56. Khokhar SK, Kommagani R, Kadakia MP. Differential effects of p63 mutants on transactivation of p53 and/or p63 responsive genes. Cell Res 2008; 18:1061-73.
57. Rinne T, Hamel B, van Bokhoven H, Brunner HG. Pattern of p63 mutations and their phenotypes—update. Am J Med Genet A 2006; 140:1396-406.
58. Rother K, Johne C, Spiesbach K, Haugwitz U, Tschöp K, Wasner M, et al. Identification of Tcf-4 as a transcriptional target of p53 signalling. Oncogene 2004; 23:3376-84.
59. Krause K, Wasner M, Reinhard W, Haugwitz U, Lange-zu Dohna C, Mössner J, Engeland K. The tumour suppressor protein p53 can repress transcription of cyclin B. Nucleic Acids Res 2000; 28:4410-8.
60. Contente A, Dittmer A, Koch MC, Roth J, Dobbelstein M. A polymorphic microsatellite that mediates induction of PIG3 by p53. Nat Genet 2002; 30:315-20.
61. Boyd KE, Wells J, Gutman J, Bartley SM, Farnham PJ. c-Myc target gene specificity is determined by a post-DNA binding mechanism. Proc Natl Acad Sci USA 1998; 95:13887-92.