Spontaneous Tumor Formation in *Trp53*-deficient Epidermis Mediated by Chromosomal Instability and Inflammation

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Abstract. *Background: The specific ablation of Trp53 gene in mouse epidermis leads to the spontaneous development of aggressive squamous cell carcinoma, a process that is accelerated by the subsequent loss of Rb gene. Materials and Methods: The possible mechanisms leading to spontaneous tumor formation in epidermis in the absence of Trp53 were studied focusing on hair cycle defects, inflammation and possible chromosomal instability (CIN). Results: Loss of p53 induces tumorigenesis primarily by mediating early CIN and, to a minor extent, nuclear factor κB activation. Notably, CIN occurs not only in p53-deficient skin, but also in epidermis lacking both Rb and Tp53 tumor suppressors, indicating a predominant role of this process in spontaneous tumorigenesis. Conclusion: These data identify CIN as a major mechanism in tumorigenesis originated by Trp53 loss in stratified epithelia and imply that therapies aimed to counterbalance CIN might be of relevance for the treatment of human cancer bearing impaired p53 functions.*

The p53 tumor suppressor coordinates the cellular response to stress, including DNA damage, hypoxia and oncogenic stress through transcriptional mechanisms, resulting in cell cycle arrest, senescence, or apoptosis. Accordingly, *p53* mutations are widely involved in human tumorigenesis (1). Moreover, altered p53 functions are also associated with poor prognosis and high metastatic potential in human tumors (1, 2).

We and others have previously described that the specific ablation of *p53* gene in stratified epithelia (*Tp53*^{lox};K14*cre* hereafter *Tp53*$^{\Delta EC}$ mice) leads to spontaneous squamous cell carcinoma (SCC) development (3, 4), in a process that is accelerated by the subsequent ablation of *Rb* gene (4). Detailed analysis indicates that such acceleration, characterized by earlier tumor development, is mediated by increased activation of Akt pathway, resulting in increased proliferation and augmented tumor angiogenesis (4). Nonetheless, the mechanisms leading to spontaneous tumor development by *Tp53* loss in epidermis remain largely unknown. To gain some insight into these mechanisms, we recently performed functional genomic analysis of tumors arising in mice bearing the epidermal deletion of *Trp53* or *Rb* and *Trp53* (5). These studies revealed that these mouse tumors share relevant characteristics with multiple human malignancies, distinguished by poor prognosis, altered *p53* status and high metastatic potential. Of note, these genomic alterations, which are independent of the *pRb* status, also revealed a primary involvement of cell cycle genes, in particular those implicated in mitosis, and a functional enrichment of stem cell-associated genes (5), in agreement with the hair follicle origin of the tumors (4).

Here we have performed a detailed analysis of the possible mechanisms leading to spontaneous tumor development in *Tp53*$^{\Delta EC}$ and their possible metastatic characteristics.

Materials and Methods

Mice and genotyping. Mice of the different genotypes were kindly provided by Dr. A Berns (NKI, Amsterdam, the Netherlands) and they were enriched in FVB background by backcrossing (4). The different mice used in this study (*Rb*$^{\Delta EC}$ (n=20), *Tp53*$^{\Delta EC}$ (n=43) and *Rb*$^{\Delta EC}$;*Tp53*$^{\Delta EC}$ (n=65)) were genotyped as previously described (4). Mice of all genotypes were monitored for tumor development over a period of 20 months and sacrificed for histopathological analyses when animals showed obvious tumors or morbidity. *Rb*$^{\Delta EC}$ and control mice were subjected to a DMBA/TPA chemical carcinogenesis protocol as described elsewhere (6). The animal experiments were approved by the Animal Ethical Committee and conducted in compliance with Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT) Guidelines.

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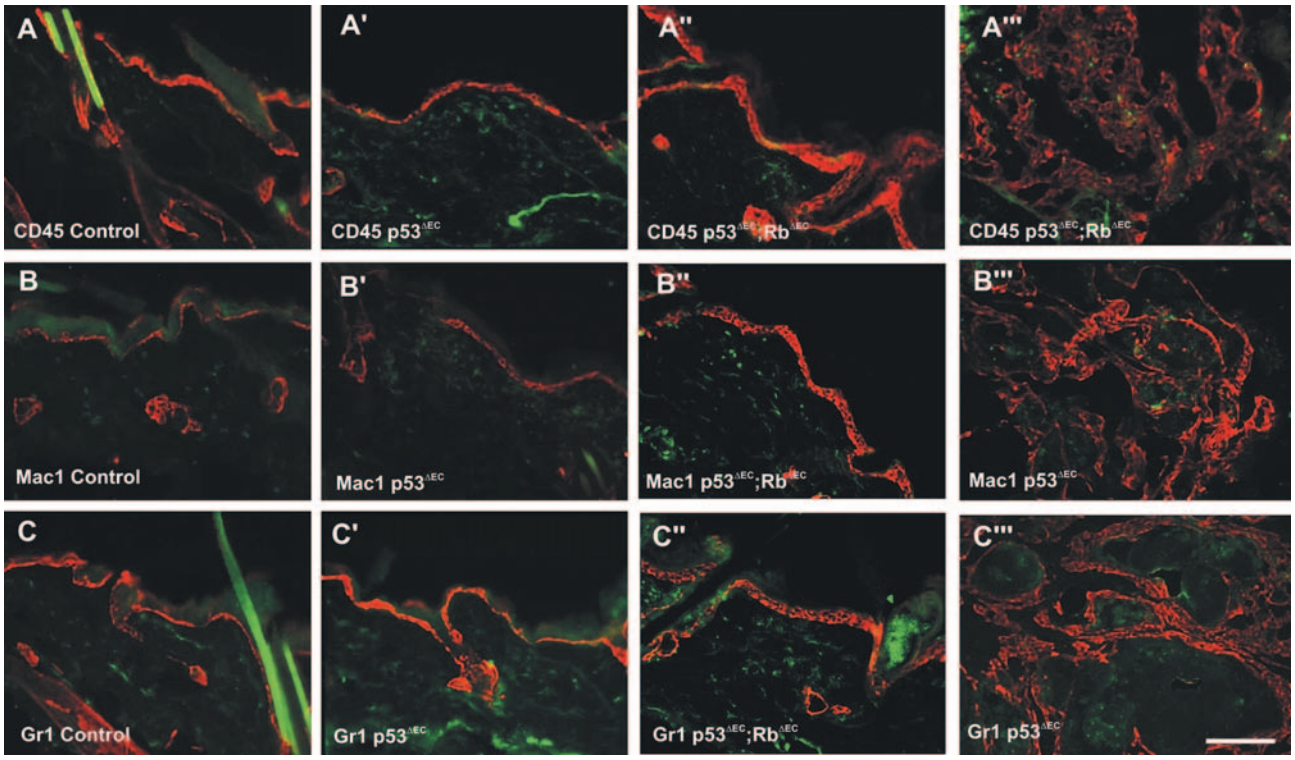

Figure 1. *Detection of inflammatory cells in frozen skin and tumor sections by double immunofluorescence with K5 (red) and CD45 as a leukocyte marker (green A), Mac-1 as a macrophage marker (green B) and Gr-1 as a granulocyte marker (green C) in control (A, B, C), $Tp53^{\Delta EC}$ (A´, B´, C´), $Rb^{\Delta EC}$;$Tp53^{\Delta EC}$ (A´´, B´´, C´´) skin and tumors (A´´´, B´´´, C´´´). An increase of the three markers is apparent in $Tp53^{\Delta EC}$ and $Rb^{\Delta EC}$;$Tp53^{\Delta EC}$ skin. No further increase was observed in tumors. Bar=150 μm.*

Histological procedures. For histological analysis, skin and tumoral samples were fixed in formalin and embedded in paraffin prior to sectioning, or were snap frozen in liquid nitrogen and embedded in OCT for cryosectioning. Sections of 5 μm were cut and stained with hematoxylin and eosin (H&E) for pathology assessment. Paraffin sections were deparaffinized. All sections were incubated with 5% horse serum for 30 minutes to block the Fc receptor in tissue, and then washed three times with sterile phosphate-buffered saline (PBS) (pH 7.5) prior to incubation with the appropriate primary antibodies diluted in PBS/bovine serum albumin (BSA). Antibodies were used as follows: in frozen sections 1/50 dilution of FITC-mouse anti-mouse CD45.2 (Ly 5.2) (BD Pharmingen, NJ, USA) for CD45 detection, fluorescein isothiocyanate (FITC)-rat anti-mouse CD11b (BD Pharmingen) for macrophage detection, FITC-rat anti-mouse Ly-6G and LY6C (BD Pharmingen) for granulocyte detection, and 1/1,000 dilution of anti-K5 (Covance, NJ, USA) for keratin 5 detection. In paraffin sections: 1/200 dilution of anti-γ-tubulin-centrosome marker (Abcam); 1/100 dilution of anti-α-tubulin for microtubule detection (Sigma, MO, USA); 1/50 dilution of P-ChK1 (S345) (Abcam) and P-Chk-2 (T68) (Abcam, Cambridge, UK). FITC- or TexasRed-conjugated secondary antibodies for immunofluorescence were purchased from Jackson ImmunoResearch (CA, USA) and used at 1/50 and 1/500, respectively. Diamidinophenylindole (DAPI) was used to counterstain the nuclei or chromosomes. Control slides were obtained by replacing primary antibodies with PBS (data not shown). The quantitative analyses of mitosis were performed using H&E-stained sections from at least five different mice/tumors scoring three to seven different sections. The total number of mitoses scored ranged from 150 to 500 for each data point. Double immunofluorescence stained sections against γ- and α-tubulin scoring at least 150 mitotic Figures for each data point. Data are shown as mean±SD.

Affymetrix mouse gene chip 430A analysis. Skin samples from newborns were preserved in RNAlater (Ambion, CA, USA) and disrupted and homogenized using Mixer Mill MM301 (Retsch, Haan, Germany). Total RNA was extracted and purified from 30 mg of skin using RNeasy Fibrous Tissue Mini kit (Qiagen, Venlo, the Netherlands) following the manufacturer's recommendations. The integrity of the RNA populations was tested in a Bioanalyzer (Agilent, CA, USA) showing an at least 1.4 28S to 18S ratio. Total RNA was hybridized at the Genomic Facility of the Centro de Investigación del Cáncer (Salamanca, Spain). We exported .cel files from Affymetrix GCOS software, and used the GEPAS Analysis Suite (7) to perform background subtraction with RMA (8), normalizing the chips using the quantile method (9) and log2 transformed and mean-centered the intensity values. ANOVA analysis ($p<0.01$), performed using MeV software (10), was used to select 1,435 genes differentially expressed among the quoted genotypes and control mice. The whole data sets are available at Gene Expression Omnibus database (GSE11990). The inflammation-related genes shown in Figure 2 were obtained by Gene Ontology using the DAVID Functional Annotation tool.

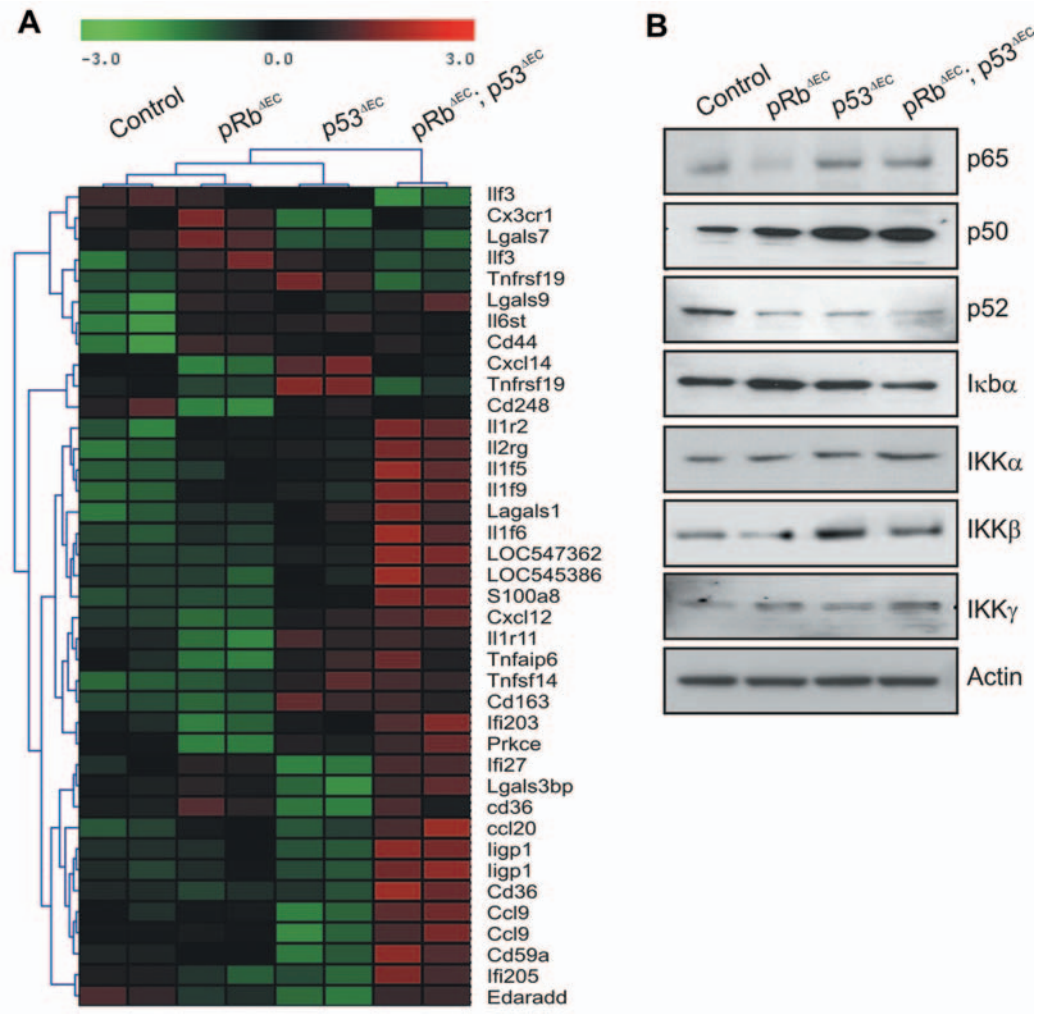

Figure 2. *A, Heat map representation of inflammation-related genes with differential expression in the quoted genotypes. B, Western blot analyses from non lesional skin samples of control, $Rb^{\Delta EC}$, $Tp53^{\Delta EC}$ and $Rb^{\Delta EC}$;$Tp53^{\Delta EC}$ mice showing increased activation of the NFκB pathway in Trp53-deficient mice.*

Western blot analysis. Whole skin extracts were ground with a mortar on liquid nitrogen, homogenized and lysed by freeze-thawing cycles in lysis buffer C (200 mM HEPES pH 7.9, 25% glycerol, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 mM PMSF, 20 mM NaF, 1 mM NaPPi, 1 mM Na_3VO_4, 2.5 mM DTT), and centrifuged to obtain supernatants containing total protein. 35μg protein per sample were resolved in SDS-PAGE gels and transferred to nitrocellulose membranes (Amersham, UK). Membranes were blocked with 5% non-fat milk diluted in TBS and incubated with the appropiate antibodies diluted in TBS-T 0.5% BSA. Antibodies were used as follows: anti-NFκB p65, anti-NFκB p50, anti-NFκB p52, anti-IKKα, anti-IKKγ all diluted 1/250 and purchased from Santa Cruz; anti-IκBα diluted 1/200 (Santa Cruz); anti-IKKβ diluted 1/1,000 (IMGenex, CA, USA); anti-actin diluted 1/100 (Santa Cruz). Secondary antibodies were purchased from Jackson ImmunoResearch and used at 1/5,000. Super Signal West Pico Chemiluminscence Substrate (Pierce, IL, USA) was used according to the manufacturer's recommendations to visualize the bands.

Results

The absence of *Trp53* in stratified epithelia through cre-LoxP system ($Tp53^{\Delta EC}$ mice) leads to spontaneous tumor formation in mice through as yet unknown mechanisms. To investigate the possible process involved in tumorigenesis, we focused on three previously reported activities of p53, which are also involved in skin tumorigenesis. Firstly, p53 is involved in hair cycle through catagen promotion (11); secondly, p53 represses inflammatory response by interfering with NFκB-dependent transcription (12), and thirdly, p53 prevents centrosome amplification and thus mitotic aberrations (13).

The possible involvement of p53 in tumorigenesis through interference with hair cycling would imply that processes leading to accelerated hair regrowth would also account for

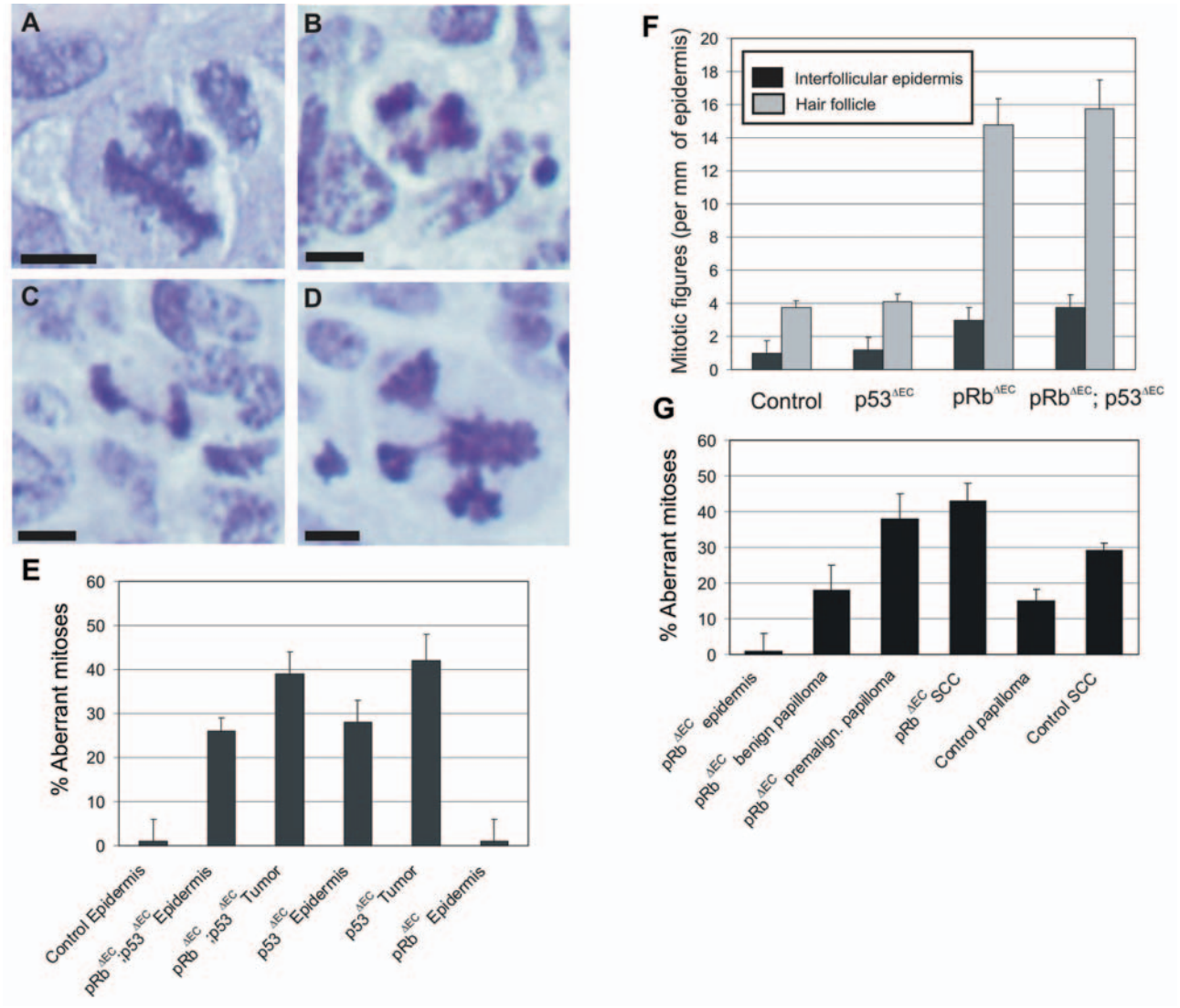

Figure 3. *Hematoxylin-eosin stainings of Trp53$^{\Delta EC}$ and Rb$^{\Delta EC}$;Trp53$^{\Delta EC}$ mouse epidermis (A, B) and tumors (C, D) respectively, showing aberrant mitosis. E, Quantification of the percentage of aberrat mitoses in epidermis (control, Rb$^{\Delta EC}$, Trp53$^{\Delta EC}$ and Rb$^{\Delta EC}$;Trp53$^{\Delta EC}$) and tumors (Tp53$^{\Delta EC}$ and Rb$^{\Delta EC}$;Tp53$^{\Delta EC}$). F, Number of mitotic figures in interfollicular epidermis and hair follicles in the quoted genotypes. G, Quantification of the percentage of aberrant mitoses in Rb$^{\Delta EC}$ epidermis and papillomas and squamous cell carcinomas in control and Rb$^{\Delta EC}$ treated with DMBA/TPA. Bars=10 μm.*

increased tumor development. To test this hypothesis, we performed repeated depilation and monitored tumor incidence in the treated areas. No increase in the number of tumors was recorded in mice (n=10) under repeated depilation after 4 months compared with untreated mice or with depilated controls (not shown), thus excluding hair cycle alterations as a main mechanism for tumor development in *Tp53*$^{\Delta EC}$ mice.

To analyze the involvement of inflammatory processes in tumor development, we monitored the presence of inflammatory cells in non-lesional epidermis of control, *Tp53*$^{\Delta EC}$ and *Tp53*$^{\Delta EC}$;*Rb*$^{\Delta EC}$ adult mice, as these two mouse models display high incidence of spontaneous tumor development in which the presence of such cells is a common characteristic (4). We observed an increased number of lymphocytes (CD45-positive), macrophages (Mac-1-positive) and granulocytes (Gr-1-positive) in *Tp53*$^{\Delta EC}$ mouse epidermis (Figure 1A´, B´, C´), compared to control epidermis (Figure 1A, B, C). Such increased numbers were not further augmented in *Tp53*$^{\Delta EC}$;*Rb*$^{\Delta EC}$ skin (Figure 1A´´, B´´, C´´) and only augmented to a moderate extent in epidermal tumors (Figure 1A´´´, B´´´, C´´´). These data suggest that the absence of *Trp53* is sufficient to elicit a proinflammatory response and recruit effector cells in epidermis prior to tumor development.

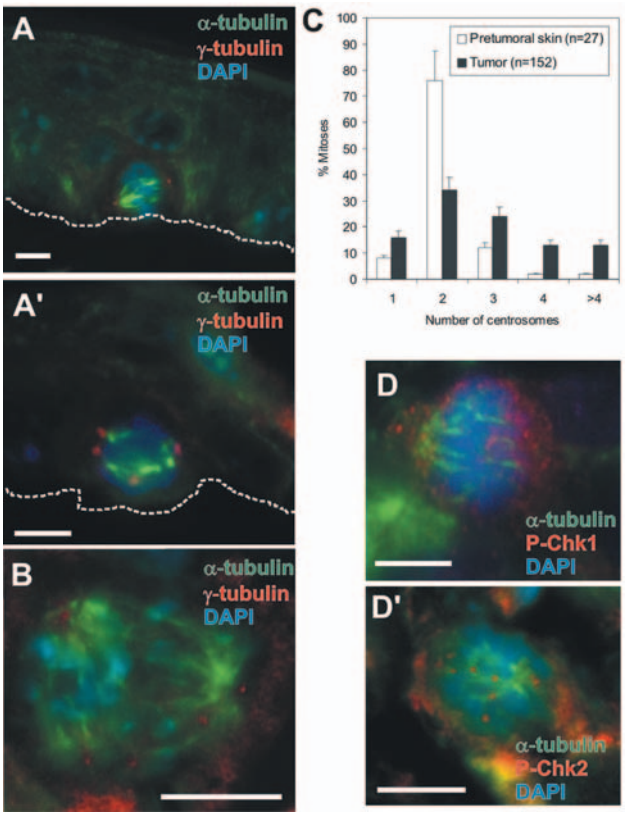

Figure 4. *Mitotic centrosomes in Tp53$^{\Delta EC}$ epidermis, showing normal (A) and abnormal (A´) centrosome numbers, and Tp53$^{\Delta EC}$ tumors (B). C), Quantification of the percentage of mitoses referred to the number of centrosomes in pretumoral skin and tumors (n denotes the total number of mitoses scored). Detection of the phosphorylated mitotic checkpoint proteins P-Chk-1 (D) and P-Chk-2 (D´) (red) by double immunofluorescence with α-tubulin (green). DAPI was used to counterstain nuclei and chromosomes. Bars=10 μm.*

To further corroborate these results, we analyzed microarray data obtained from newborn mouse epidermis (4) in search for possible pro-inflammatory genes. This analysis (Figure 2A) indicated that there is a reduced number of genes that display increased expression in *Tp53*$^{\Delta EC}$ exclusively, whereas the vast majority of them display a pattern characterized either by augmented expression in *Tp53*$^{\Delta EC}$;*Rb*$^{\Delta EC}$ mouse epidermis, or by a moderate increase in *Tp53*$^{\Delta EC}$ further increased in *Tp53*$^{\Delta EC}$;*Rb*$^{\Delta EC}$. These data indicate that the absence of *Trp53* or, more importantly, the simultaneous absence of *Rb* and *Trp53*, promote expression changes in multiple inflammation-related genes. Finally, given the relevance of the NFκB pathway in regulating the expression of inflammatory genes, we analyzed the expression of proteins belonging to this pathway in mouse epidermis. We observed (Figure 2B) an increase in p65, p50 and IKKβ in *Tp53*$^{\Delta EC}$ and *Tp53*$^{\Delta EC}$;*Rb*$^{\Delta EC}$ samples, whereas no significant changes were observed in IKKα. Of note, the expression of p52 decreased in all transgenic samples, and a moderate increase in IKKγ and a moderate decrease in IκBα was only observed in *Tp53*$^{\Delta EC}$;*Rb*$^{\Delta EC}$ mouse epidermis. Overall, these data indicate that the absence of p53 in epidermis triggers a pro-inflammatory response which is only partially dependent on NFκB activation.

Since mutations in *p53* promote mitotic aberrations which may lead to spontaneous tumor development (14-16), we studied the presence of aberrant mitosis in *Tp53*$^{\Delta EC}$ and *Tp53*$^{\Delta EC}$;*Rb*$^{\Delta EC}$ mouse epidermis and tumors. Multiple mitotic aberrations were observed in epidermis (Figure 3A, B, E) and in epidermal tumors (Figure 3C, D, E), suggesting that deregulated mitotic progression could be responsible for spontaneous tumor development. We previously reported that tumors arising in *Tp53*$^{\Delta EC}$ and *Tp53*$^{\Delta EC}$; *Rb*$^{\Delta EC}$ mice display a clear hair follicle origin (4), since proliferation, and thus mitosis, is augmented in these areas compared with interfollicular epidermis (Figure 3F), this may help to explain our previous data. Moreover, proliferation in hair follicles is dramatically increased by the loss of *Rb* (Figure 3F), which might act synergistically with *Trp53* loss to increase the number of mitotic aberration in *Tp53*$^{\Delta EC}$;*Rb*$^{\Delta EC}$ hair follicles, thus providing further support for the accelerated tumor formation observed in *Tp53*$^{\Delta EC}$;*Rb*$^{\Delta EC}$ mice (4). Finally, to further support the involvement of *Trp53* loss in promoting mitotic aberrations in epidermis and thus favoring tumor development, we also monitored possible alterations in papillomas and SCCs arising upon chemical carcinogenesis protocols in *Rb*$^{\Delta EC}$ epidermis (6). In this system, the development of papillomas is prevented by overactivation of p53-dependent pathways, leading to premature p53 loss of function and increased malignant conversion of papillomas in SCCs (6). *Rb*-deficient malignant papillomas, which have lost p53 functions (premalignant), displayed a significant increase of aberrant mitosis, similar to that observed in *Rb*-deficient SCCs and exceeding that shown by SCCs developed in control mice (Figure 3G).

One of the control mechanisms exerted by p53, affecting mitotic control, is the centrosome duplication (13, 17), and multiple multipolar mitoses were observed in our analysis (Figure 3B-D). Consequently, we monitored centrosome number in epidermis and tumors of *Tp53*$^{\Delta EC}$ mice. Although the majority of mitoses in non-tumoral epidermis displayed a normal number of centrosomes (Figure 4A, C), we detected an increased number of centrosomes in both non-tumoral epidermis and epidermal tumors in *Tp53*$^{\Delta EC}$ mice (Figure 4A´, B, C). These observations indicate that the altered mitoses in *Tp53*$^{\Delta EC}$ mouse epidermis and tumors were mediated, at least in part, by aberrant centrosome duplication and positioning. Importantly, such altered centrosome number was associated with increased activation of mitotic

checkpoints characterized by augmented expression of phosphorylated Chk1 and Chk2 (Figure 4D, D´, respectively), in agreement with our previous findings of increased expression of mitosis controlling genes in tumors arising in *p53*-deficient epidermis (5).

Collectively, the data presented here support the hypothesis that the spontaneous tumor development due to *p53* absence is mediated by premature mitotic aberrations.

Discussion

The *p53* gene is one of the most frequently mutated tumor suppressor genes in human malignancies, and its loss of function is associated with poor prognosis and high metastatic potential in multiple human tumors (1, 2). As a consequence, numerous efforts are directed to target the pathways affected by *p53* loss as promising therapeutic alternatives (18). Such efforts require the use of well suited preclinical models to asses the efficiency of such therapies in an *in vivo* setting. We and others have previously reported the development of SCC in $Tp53^{\Delta EC}$ mice (3, 4), and the collaboration of the subsequent ablation of *Rb* gene in the process (4). Moreover, functional genomic analysis of the tumors compared with control epidermis revealed that the loss of *p53* is the most relevant characteristic for tumor development, and that the molecular changes allowed the identification and classification of human tumors harboring *p53* mutations, poor prognosis and high metastatic behavior (5). Consequently, these mice represent an attractive alternative for testing molecularly targeted therapies. In spite of this, the molecular bases of tumor development in $Tp53^{\Delta EC}$ mice are not well characterized. Here we focused on the three major mechanisms that might be relevant in the context of epidermis without external carcinogenic stimuli.

It is well assumed that most epidermal tumors arise in the hair follicle, probably in close relationship with epidermal stem cells (19, 20). Since p53 protein is suggested to act during catagen induction, the regression phase of the hair cycle (11), one may expect possible altered hair cycle and increased tumorigenesis in $Tp53^{\Delta EC}$ mice upon repeated depilation. However, although spontaneous tumors arising in $Tp53^{\Delta EC}$ mice display a clear hair follicle origin (4), we did not find tumor development in the treated areas, thus excluding the possibility that hair cycle alterations resulted in increased tumorigenesis. This can be explained if the functions of p53 can be carried by other proteins (21) and/or in the context of complete p53 loss, affecting epidermal and dermal cells (11), these functions can be discerned.

It has been reported that mutated *p53* expression correlates positively with NFκB activity in cultured cancer cells and that mutant p53 can elevate *NFκB* activity *via* transactivation of the *NFκB2* gene, in particular in the context of SCC development (12, 22-25). Our data add the functional characterization of proinflammatory cells prior to tumor development in mice bearing the epidermal specific deletion of *Trp53* (Figure 1). Furthermore, in skin of newborns we found the expression of multiple genes involved in inflammatory response and deregulation of NFκB pathway proteins (Figure 2). These data highlight the possibility that inhibitors of inflammation, such as COX-2 inhibitors (26, 27) can be of use as anticancer therapies in mutant *p53*- bearing tumors.

Finally, we also analyzed possible premature chromosome instability in $Tp53^{\Delta EC}$ mouse. Chromosomal instability is an early event in SCC development (28-30) and the functions of p53 mediating proper mitotic progression are well recognized (31-33). Therefore it is not surprising that the mutations in *p53* promote mitotic aberrations leading to spontaneous tumor development (14, 15), which might be increased by *Rb* family inactivation (16). Overall, our findings are in agreement with these observations, as we detected frequent aberrant mitoses in $Tp53^{\Delta EC}$ mouse tumors. Moreover, we also describe that such mitotic aberrations are frequent events prior to overt tumor development, thus reinforcing the oncogenic functions of chromosome instability. The observations that centrosome numbers are also altered prior to tumor formation also indicates that the functions of p53 controlling such process are essential for tumor suppressor activities. However, our data also seem to be in disagreement with the absence of centrosome aberrations in skin tumors promoted by *Kras* activation in $Tp53^{\Delta EC}$ mice (14). Such apparent discrepancy can be explained by the use of an inducible cre system, the presence of Kras expression, or the induction of tumor formation by TPA treatment used by other authors (14). Indeed, the spontaneous tumor development between $Tp53^{\Delta EC}$ and mice expressing the *p53*.R270H mutation is similar, and the differences are only appreciable upon UV carcinogenic stimuli (34).

Our data may also suggest that chromosome instability by complete loss of function, either by *Trp53* loss or dominant functions, may represent a more dramatic effect than abrogating some specific p53 functions in oncogenesis. Indeed, the chromosomal instability observed in $Rb^{\Delta EC}$ mice upon two-stage carcinogenesis leading to aggressive and poorly differentiated carcinomas (6, 35) is in contrast with the poorly aggressive and highly differentiated tumors arising in $Rb^{\Delta EC}$;$p107^{-/-}$ mice (36), in which the absence, or reduced expression, of p107 abrogates the proapoptotic functions of p53 without affecting cell cycle (36, 37). In support of this, a chromosomal instability signature represents a potential tool in determining malignancy and metastatic behavior over a broad range of human tumors (38), and the genomic analysis of $Tp53^{\Delta EC}$ tumors, which display clear parallelism with chromosomal instability, is also able to predict malignant and metastatic behavior of several types of human cancer (5). Collectively, such observations indicate that $Tp53^{\Delta EC}$ mice can be an excellent tool for the preclinical evaluation of drugs targeted against mitotic regulators.

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References

1 Petitjean A, Mathe E, Kato S, Ishioka C, Tavtigian SV, Hainaut P and Olivier M: Impact of mutant p53 functional properties on *TP53* mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. Hum Mutat *28(6)*: 622-629, 2007.

2 Toledo F and Wahl GM: Regulating the p53 pathway: *in vitro* hypotheses, *in vivo* veritas. Nat Rev Cancer *6(12)*: 909-23.2006.

3 Jonkers J, Meuwissen R, van der Gulden H, Peterse H, van der Valk M and Berns A: Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. Nat Genet *29(4)*: 418-425, 2001.

4 Martinez-Cruz AB, Santos M, Lara MF, Segrelles C, Ruiz S, Moral M, Lorz C, Garcia-Escudero R and Paramio JM: Spontaneous squamous cell carcinoma induced by the somatic inactivation of retinoblastoma and Trp53 tumor suppressors. Cancer Res *68(3)*: 683-692, 2008.

5 García-Escudero R, Martinez-Cruz AB, Santos M, Bielza C, Segrelles C, Moral M, Lorz C, Costa C, Buitrago-Pérez Á, Saiz-Ladera C, Larrañaga P and Paramio JM: Mouse *p53*-deficient carcinoma models define molecular determinants of metastasis and predict human cancer outcome. Submitted.

6 Ruiz S, Santos M, Lara MF, Segrelles C, Ballestin C and Paramio JM: Unexpected roles for pRb in mouse skin carcinogenesis. Cancer Res *65(21)*: 9678-9686, 2005.

7 Vaquerizas JM, Conde L, Yankilevich P, Cabezon A, Minguez P, Diaz-Uriarte R, Al-Shahrour F, Herrero J and Dopazo J: GEPAS, an experiment-oriented pipeline for the analysis of microarray gene expression data. Nucleic Acids Res *33(Web Server issue)*: W616-620, 2005.

8 Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U and Speed TP: Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics *4(2)*: 249-264, 2003.

9 Bolstad BM, Irizarry RA, Astrand M and Speed TP: A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics *19(2)*: 185-193, 2003.

10 Saeed AI, Sharov V, White J, Li J, Liang W, Bhagabati N, Braisted J, Klapa M, Currier T, Thiagarajan M, Sturn A, Snuffin M, Rezantsev A, Popov D, Ryltsov A, Kostukovich E, Borisovsky I, Liu Z, Vinsavich A, Trush V and Quackenbush J: TM4: a free, open-source system for microarray data management and analysis. Biotechniques *34(2)*: 374-378, 2003.

11 Botchkarev VA, Komarova EA, Siebenhaar F, Botchkareva NV, Sharov AA, Komarov PG, Maurer M, Gudkov AV and Gilchrest BA: p53 Involvement in the control of murine hair follicle regression. Am J Pathol *158(6)*: 1913-1919, 2001.

12 Komarova EA, Krivokrysenko V, Wang K, Neznanov N, Chernov MV, Komarov PG, Brennan ML, Golovkina TV, Rokhlin OW, Kuprash DV, Nedospasov SA, Hazen SL, Feinstein E and Gudkov AV: p53 is a suppressor of inflammatory response in mice. FASEB J *19(8)*: 1030-1032, 2005.

13 Fukasawa K, Choi T, Kuriyama R, Rulong S, and Vande Woude GF: Abnormal centrosome amplification in the absence of p53. Science *271(5256)*: 1744-1747, 1996.

14 Caulin C, Nguyen T, Lang GA, Goepfert TM, Brinkley BR, Cai WW, Lozano G and Roop DR: An inducible mouse model for skin cancer reveals distinct roles for gain- and loss-of-function *p53* mutations. J Clin Invest *117(7)*: 1893-1901, 2007.

15 Wang XJ, Greenhalgh DA, Jiang A, He D, Zhong L, Medina D, Brinkley BR and Roop DR: Expression of a *p53* mutant in the epidermis of transgenic mice accelerates chemical carcinogenesis. Oncogene *17(1)*: 35-45, 1998.

16 Duensing A and Duensing S: Guilt by association? p53 and the development of aneuploidy in cancer. Biochem Biophys Res Commun *331(3)*: 694-700, 2005.

17 Fukasawa K: P53, cyclin-dependent kinase and abnormal amplification of centrosomes. Biochim Biophys Acta *1786(1)*: 15-23, 2008.

18 Lane DP: Exploiting the p53 pathway for the diagnosis and therapy of human cancer. Cold Spring Harb Symp Quant Biol *70*: 489-497, 2005.

19 Owens DM and Watt FM: Contribution of stem cells and differentiated cells to epidermal tumours. Nat Rev Cancer *3(6)*: 444-451, 2003.

20 Lorz C, Segrelles C and Paramio JM: On the origin of epidermal cancers. Curr Mol Med *9(3)*: 353-364, 2009.

21 Seiberg M, Marthinuss J and Stenn KS: Changes in expression of apoptosis-associated genes in skin mark early catagen. J Invest Dermatol *104(1)*: 78-82, 1995.

22 Ferris RL and Grandis JR: *NF-κB* gene signatures and *p53* mutations in head and neck squamous cell carcinoma. Clin Cancer Res *13(19)*: 5663-5664, 2007.

23 Weisz L, Damalas A, Liontos M, Karakaidos P, Fontemaggi G, Maor-Aloni R, Kalis M, Levrero M, Strano S, Gorgoulis VG, Rotter V, Blandino G and Oren M: Mutant p53 enhances nuclear factor κB activation by tumor necrosis factor α in cancer cells. Cancer Res *67(6)*: 2396-2401, 2007.

24 Scian MJ, Stagliano KE, Anderson MA, Hassan S, Bowman M, Miles MF, Deb SP and Deb S: Tumor-derived *p53* mutants induce NF-κB2 gene expression. Mol Cell Biol *25(22)*: 10097-1110, 2005.

25 Gulati AP, Yang YM, Harter D, Mukhopadhyay A, Aggarwal BB, Benzil DL, Whysner J, Albino AP, Murali R and Jhanwar-Uniyal M: Mutant human tumor suppressor p53 modulates the activation of mitogen-activated protein kinase and nuclear factor-κB, but not c-Jun *N*-terminal kinase and activated protein-1. Mol Carcinog *45(1)*: 26-37, 2006.

26 Benoit V, de Moraes E, Dar NA, Taranchon E, Bours V, Hautefeuille A, Taniere P, Chariot A, Scoazec JY, de Moura Gallo CV, Merville MP and Hainaut P: Transcriptional activation of cyclooxygenase-2 by tumor suppressor p53 requires nuclear factor-κB. Oncogene *25(42)*: 5708-5718, 2006.

27 de Moraes E, Dar NA, de Moura Gallo CV and Hainaut P: Cross-talks between cyclooxygenase-2 and tumor suppressor protein p53: Balancing life and death during inflammatory stress and carcinogenesis. Int J Cancer *121(5)*: 929-937, 2007.

28 Slaga TJ, O'Connell J, Rotstein J, Patskan G, Morris R, Aldaz CM and Conti CJ: Critical genetic determinants and molecular events in multistage skin carcinogenesis. Symp Fundam Cancer Res *39*: 31-44, 1986.

29 Aldaz CM, Conti CJ, Larcher F, Trono D, Roop DR, Chesner J, Whitehead T and Slaga TJ: Sequential development of aneuploidy, keratin modifications, and gamma-glutamyltransferase expression in mouse skin papillomas. Cancer Res *48(11)*: 3253-7, 1988.

30 Aldaz CM, Conti CJ, Yuspa SH and Slaga TJ: Cytogenetic profile of mouse skin tumors induced by the viral *Harvey-ras* gene. Carcinogenesis *9(8)*: 1503-1505, 1988.

31 Tomasini R, Mak TW and Melino G: The impact of p53 and p73 on aneuploidy and cancer. Trends Cell Biol *18(5)*: 244-252, 2008.

32 Fukasawa K: Centrosome amplification, chromosome instability and cancer development. Cancer Lett *230(1)*: 6-19, 2005.

33 Tarapore P and Fukasawa K: *p53* mutation and mitotic infidelity. Cancer Invest *18(2)*: 148-155, 2000.

34 Wijnhoven SW, Speksnijder EN, Liu X, Zwart E, Vanoostrom CT, Beems RB, Hoogervorst EM, Schaap MM, Attardi LD, Jacks T, van Steeg H, Jonkers J and de Vries A: Dominant-negative but not gain-of-function effects of a p53.R270H mutation in mouse epithelium tissue after DNA damage. Cancer Res *67(10)*: 4648-4656, 2007.

35 Ruiz S, Santos M and Paramio JM: Is the loss of *pRb* essential for mouse skin carcinogenesis? Cell Cycle *5(6)*: 625-629, 2006.

36 Lara MF, Santos M, Ruiz S, Segrelles C, Moral M, Martinez-Cruz AB, Hernandez P, Martinez-Palacio J, Lorz C, Garcia-Escudero R and Paramio JM: p107 acts as a tumor suppressor in *pRb*-deficient epidermis. Mol Carcinog *47(2)*: 105-113, 2008.

37 Santos M, Ruiz S, Lara MF, Segrelles C, Moral M, Martinez-Cruz AB, Ballestin C, Lorz C, Garcia-Escudero R, and Paramio JM: Susceptibility of *pRb*-deficient epidermis to chemical skin carcinogenesis is dependent on the *p107* allele dosage. Mol Carcinog *47(11)*: 815-821, 2008.

38 Carter SL, Eklund AC, Kohane IS, Harris LN and Szallasi Z: A signature of chromosomal instability inferred from gene expression profiles predicts clinical outcome in multiple human cancers. Nat Genet *38(9)*: 1043-1048, 2006.

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