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Transgenic human programmed cell death 5 expression in mice suppresses skin cancer development by enhancing apoptosis ☆

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ABSTRACT

Aims: We sought to probe the role of human programmed cell death 5 (PDCD5) in vivo and to understand its mechanisms.
Main methods: A transgenic mouse model of human PDCD5 was generated by pronuclear microinjection. Apoptosis in tissues of three independent transgenic mouse lines was quantified by terminal deoxynucleotidyl transferase mediated dUTP Nick End Labeling (TUNEL) and compared to wild type littermates. Their lifespan was compared. 8-Week PDCD5 mice and wild type mice (at a group of 5) were treated with carcinogen 3-methylcholanthrene (3-MC) at 5 μg per week to induce skin cancer. Cancer development was measured by examining hematoxylin and eosin (H&E) stained skin sections after 5 weeks and 10 weeks treatment. Protein expression was determined by Western blot and apoptosis of skin cells was quantified by TUNEL.
Key findings: Starting from 5 months after birth, significant autonomous apoptosis was observed in multiple tissues of transgenic mice including skin, liver, spleen, adrenal gland and thyroid gland comparing to their wild type littermates. The average lifespan of PDCD5 mice was reduced to 9.75 months (normally 24–30 months). Moreover, carcinogen 3-MC induced skin cancer development was attenuated in the lesion of PDCD5 transgenic mice by enhancing apoptosis. Pro-apoptotic protein Bax expression was up-regulated in the 3-MC treated skin of transgenic mice.
Significance: These results suggest PDCD5 plays an antitumor role by enhancing apoptosis in animal physiological settings. Therefore, PDCD5 is a potential target for cancer therapy.

Introduction

The induction of apoptosis in tumor cells has been a pivotal goal in cancer therapy (Dewey et al., 1995; Rupnow and Knox, 1999; Cragg et al., 2009). However, the relative influences of apoptosis on clinical cancer therapy remain unclear. Apoptotic death is also known to promote wound healing and normal tissue regeneration by stimulating stem cell proliferation (Li et al., 2010; Ryoo et al., 2004). Recently Huang et al. showed that the radiation-induced activation of caspase 3, a major mediator of apoptosis in response to stress, promotes the growth of the tumor cells that survive radiation and repopulation by arachidonic acid and prostaglandin E_2 (PGE_2) production (Huang et al., 2011). Thus, the relationship between apoptosis and the response of tumors to apoptosis induction will still need further in vivo investigation.

Human programmed cell death 5 (PDCD5) is a novel apoptosis-accelerating gene, which formerly referred to as TF-1 cell apoptosis-related gene 19 (TFAR19) (Liu et al., 1999a). PDCD5 facilitates apoptosis and enhances TAJ/TROY-induced paraptosis-like cell death (Wang et al., 2004). When it is over-expressed in multiple cancer cell lines, PDCD5 promotes apoptosis triggered by certain stimuli such as growth factor withdrawal or serum withdrawal from culture medium (Liu et al., 1999b). During apoptosis, PDCD5 expression is up-regulated and then translocates to nuclei rapidly (Chen et al., 2001). Oppositely, reducing functional intracellular PDCD5 suppresses apoptosis (Rui et al., 2002).

PDCD5 is widely expressed in normal human tissues and its mRNA is significantly lower in fetal tissues than in the adults (Liu et al., 1999b; Dobson et al., 2004). PDCD5 expression is down-regulated in human tumors, such as breast cancer, lung cancer, hepatocellular carcinoma and chronic myelogenous leukemia (Xu et al., 2001; Hedenfalk and Chen, 2001; Ruan et al., 2007; Zhang et al., 2011; Li et al., 2008; Liu et al.,

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2004). SNPs in the 5′ upstream region of PDCD5 affect promoter activity and are associated with chronic myelogenous leukemia development and lung cancer (Ma et al., 2005; Spinola et al., 2006). These observations suggest decreased PDCD5 expression may be one of the mechanisms of tumor development. Human PDCD5 protein has antitumor activities in vitro. It was recently reported that recombinant human PDCD5 protein combined with chemotherapy drugs had antitumor activities in chronic myelogenous leukemia K562 cells (Shi et al., 2010). Adenovirus carrying PDCD5 gene exerted an antitumor efficacy on human leukemic cell lines (Xie et al., 2009). Introducing PDCD5 into cancer cells in these studies all enhances cell apoptosis. These in vitro studies suggest PDCD5 is a potential target of cancer treatment.

To date, the in vivo apoptosis-promoting and tumor suppressing functions of PDCD5 are still unknown. Here we reported a transgenic mouse model of PDCD5 by pronuclear microinjection. We found that PDCD5 transgenic mice significantly increased apoptosis cells comparing to their wild type littermates; PDCD5 expression in transgenic mice attenuated carcinogen 3-MC induced skin cancer development and delayed the onset of skin tumor in vivo. Our results suggest that PDCD5 is a potential gene target which will benefit cancer therapy.

Materials and methods

Ethics statement

All animal experiments were done according to the License for Performing Animal Experiments of Beijing, which is approved and required by the Ethics Committee of Peking University Health Science Center. The permit number is 04941.

Generation of transgenic mice

A 2.5 kb *Bgl* II and *Stu* I linearized fragment was released from pcDNA3.1-hPDCD5-Myc plasmid containing a CMV promoter, PDCD5 cDNA, a Myc tag and a 3′ poly A. The fragment is purified for pronuclear injection. The generation of PDCD5 transgenic mice was performed using the standard method (Marten and Hofker, 2002). Genomic DNA was extracted from tail snips at 3 weeks of age. Primers (forward: TAGGCGTGTACGGTGGGAGG; reverse: CGACGGCGCTATTCAGATC) were used to screen the presence of the transgene by PCR.

Fluorescence in situ hybridization (FISH)

Mice were injected with colchicine (0.4 mg/g body weight) 3 hours before sacrificed. Bone marrow cells were prepared on slides. FISH probe was synthesized by random priming labeling using DIG High Prime DNA Labeling and Detection Starter Kit (Roche). Briefly, 250 ng of DIG-labeled probe was added to each slide, denatured for 10 min at 80 °C, and then hybridized at 37 °C overnight. After washing in 2× SSC/0.2% Tween 20, slides were incubated in series with anti-DIG solution, anti-mouse-IgG-DIG and anti-DIG-fluorescein and counterstained with DAPI.

TUNEL

TUNEL assay was performed in tissue sections using One Step TUNEL Apoptosis Assay Kit (Beyotime Biotechnology Co.) principally according to the manufacturer's instructions. Briefly, tissue sections were de-paraffinized, re-hydrated followed by digestion with DNase and proteinase K (20 μg/ml) for 20 minutes at 37 °C. Slides were incubated with TUNEL detection kit for 60 minutes at 37 °C. TUNEL specimens were observed under the fluorescence microscope (Olympus, Japan) to detect the apoptosis signal intensity.

3-MC induced skin cancer, H&E staining

3-MC was spread on the dorsal skin of mice weekly for 10 weeks at 5 μg per treatment. At 5 weeks and 10 weeks, mice were sacrificed and their treated skin tissues were paraffin embedded and stained with hematoxylin and eosin. In the second 3-MC experiment, mice were treated with 3-MC at 10 μg per week until skin tumor was induced at 5 weeks.

Western blot

Total proteins were extracted and quantified following the protocols of the manufacturer (ApplyGen). Equal proteins were subjected to SDS-PAGE and transferred to nitrocellulose membrane. Western blot analyses were performed by a standard method with antibodies to hPDCD5, anti-Bax and anti-β-actin (Zhongshan Golden Bridge Biotechnology Co.).

Statistical analysis

Student's *t*-test was used to compare differences between groups. A difference was taken as significant when a *P* value was less than 0.05.

Results

Generation of PDCD5 transgenic mice

Linear DNA fragments containing a CMV promoter, PDCD5 cDNA, a Myc tag and a 3′ termination polyadenylation site were microinjected into 420 fertilized eggs (Fig. 1A). Total 67 offspring were obtained and 6 independent founders were identified by PCR (Fig. 1B). The transgene genomic integration rate was 9% (Table 1). PDCD5 transgene integration was further confirmed by fluorescence in situ hybridization (FISH) (Fig. 1C). The transgene was successfully transmitted from founder mice to their offspring.

Increased apoptosis and decreased lifespan of PDCD5 transgenic mice

Five months after birth, PDCD5 mice were observed hair loss, skin congestion and exudation on their necks and upper arms. Of the six transgenic mouse lines, we further investigated No. 6, 21 and 38 which displayed most obvious skin phenotypes. H&E staining sections of affected skin from PDCD5 mice showed abundance of apoptotic bodies, condensed chromatin accumulated along nuclear margin (data not shown). Further investigation by TUNEL staining found significantly increased apoptosis signals in multiple tissues and organs of the three transgenic mouse lines, excluding the transgene positional effect caused phenotypes. Typical images were shown from the skin of founder mice no. 38 (Fig. 2A), the thyroid gland of founder mice no. 6 (Fig. 2B) and the spleen of one offspring of founder mice no. 21 (Fig. 2C). In contrast, less apoptotic changes were observed in their wild type littermates (Fig. 2A–C). PDCD5 expression was detected in above tissues of transgenic mice but not wild type, suggesting the correlation of PDCD5 expression and increased apoptosis (Fig. 2D).

Shortened lifespan in PDCD5 mice was observed. Comparing to wild type mice which lived up to 24–30 months, PDCD5 mice only survived 9.75 months on average, based on the lifespan statistics from 24 mice of F_0–F_5 generation (Fig. 3).

PDCD5 delayed carcinogen 3-methylcholanthrene (3-MC) induced skin cancer development in transgenic mice

To explore whether PDCD5 functions as a tumor suppressor in vivo, we treated 8 weeks old PDCD5 mice of mouse line no. 38 and wild type mice (5 mice in a group) with 3-MC, which was a known chemical