Expression of p53 during mouse embryogenesis

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Summary

By in situ hybridisation we have examined the expression of p53 during mouse embryogenesis from day 8.5 to day 18.5 post coitum (p.c.). High levels of p53 mRNA were detected in all cells of the day 8.5 p.c. and 10.5 p.c. mouse embryo. However, at later stages of development, expression became more pronounced during differentiation of specific tissues e.g. of the brain, liver, lung, thymus, intestine, salivary gland and kidney. In cells undergoing terminal differentiation, the level of p53 mRNA declined strongly. In the brain, hybridisation signals were also observed in postmitotic but not yet terminally differentiated cells. Therefore, gene expression of p53 does not appear to be linked with cellular proliferation in this organ. A proposed role for p53 in cellular differentiation is discussed.

Key words: mouse embryogenesis, p53, in situ hybridisation.

Introduction

It is probable that proto-oncogenes have important roles in regulation of cellular proliferation and/or differentiation since it is well-known that growth deregulation effects are produced by their transforming counterparts. In this respect, proto-oncogenes might well be active during embryonic development. During the past few years, the in situ hybridisation technique has become a powerful method to describe the temporal and spatial expression patterns of various proto-oncogenes during embryogenesis and therefore has helped to identify their functions during normal developmental processes. Some proto-oncogenes, such as wnt-1, wnt-2 (formerly int-1 and int-2), c-fms, c-myc, N-myc, c-jun and jun B, are expressed in specific temporal and spatial patterns during mouse development and may be involved in embryonic proliferation and differentiation processes (Wilkinson et al. 1987, 1988; Regenstreif and Rossant, 1989; Schmid et al. 1989; Mugrauer et al. 1989; Wilkinson et al. 1989).

p53, a cellular phosphoprotein so called because of its molecular weight on SDS-polyacrylamide gels, was long considered to be an oncogene. However, recently it became apparent that only mutant p53 has an oncogenic potential whereas wild-type p53 must be considered as a product of a tumor suppressor gene (for a review see: Lane and Benchimol, 1990; Levine and Momand, 1990).

Certain cell types transformed by different agents such as RNA and DNA-tumor viruses or by chemical carcinogens express higher levels of p53 protein compared to non-transformed cells (Lane and Crawford, 1979; Linzer and Levine, 1979). The marked differences between the levels of p53 found in normal and transformed cells can largely be explained by an increased stability of the p53 gene product (Oren et al. 1981; Reihsaus et al. 1990). An analysis of cells in culture has suggested that p53 protein levels and the conformation of the protein appear to change during cell division and proliferation (Milner and Milner, 1981; Shohat et al. 1987; Milner and Watson, 1990).

During embryogenesis, levels of p53 protein seem to be regulated by differential gene expression as well as at the post-transcriptional level (Oren, 1985). Louis et al. (1988) reported a decline in the steady-state level of the p53 protein and a decline in the p53 mRNA during embryonic development of mouse and chicken. Nuclear run-on experiments revealed that the decrease in the steady-state levels of the p53 mRNA is not caused by a decrease in the rates of transcription but is more likely due to altered post-transcriptional modifications.

To date nothing is known about the regulation of p53 expression in defined organs during embryogenesis. To address this issue, we have examined in this study p53 gene expression during mouse embryogenesis by in situ hybridisation.

Materials and methods

Sample preparations

Embryos, placentas and whole deciduas were isolated from RB(4.15)4Rma mice (Jackson Laboratories) at the times
indicated in the text. Midday of the day of vaginal plug appearance was considered as day 0.5 post coitum (p.c.). Samples were fixed overnight at 4°C in a freshly prepared solution of 4% paraformaldehyde in PBS and were then placed overnight at 4°C in 0.5 M sucrose in PBS before storage in liquid nitrogen. Prior to sectioning embryos were embedded in OCT compound (Miles). 10 μm cryostat sections were placed on 3-amino-propyltrimethoxysilane-treated slides (Rentsch et al. 1986) and stored at −70°C. Prior to hybridisation with RNA probes, the sections were dried for 5 min on a heating plate at 50°C, postfixed with 4% paraformaldehyde in PBS for 5 min, rinsed in PBS and H2O2, depurinated for 20 min with 0.2 M HCl at room temperature, treated for 30 min with 2×SSC at 70°C, dehydrated with increasing ethanol solutions and finally air dried. All solutions were treated with 0.1% diethylpyrocarbonate and autoclaved.

**Preparation of probes**

‘Sense’ and ‘antisense’ RNA probes were labelled with α35S-UTP (1200 Ci mmol−1, New England Nuclear) to a specific activity of >106 disintegrations min−1 μg−1 using SP6 or T7 RNA polymerase and according to the suppliers directions (Boehringer Mannheim). The p53 riboprobe template was a ~1200 nucleotide long Spm—BglII mouse p53 c-DNA fragment from P53p17 (Pinhasi and Oren, 1984), subcloned into PGEM-3Z (Promega, Biotec). The full-length riboprobes were subsequently reduced to 100–200 bases in length by partial hydrolysis (Cox et al. 1984).

**In situ hybridisation**

Prehybridisation was performed at 54°C for 3 h in 50% formamide, 10% dextran sulfate, 0.3% NaCl, 10 mM Tris, 10 mM sodium phosphate pH6.8, 20 mM dithiothreitol, 0.2 mM Denhardt's reagent, 0.1 mg ml−1 E. coli RNA and cold 0.2 μM α35S-UTP. Hybridisation was carried out overnight in the same mix supplemented with 1×105 cts min−1 μl−1 of α35S-UTP-labelled RNA probe in a humidified chamber at 54°C. Slides were washed in hybridisation solution without dextran sulfate, RNA and cold UTP containing 50% formamide and 10 mM dithiothreitol at 55°C two times for 1 h and equilibrated for 15 min in a buffer solution consisting of 0.5 M NaCl, 10 mM Tris, 1 mM EDTA pH7.5. Sections were then washed with 2×SSC at 1 h and then in 0.1×SSC for 1 h at 37°C. Sections were then sequentially dehydrated in 65%, 85% and 95% (v/v) ethanol solutions containing 300 mM ammonium acetate and absolute ethanol before being air dried. Following X-ray autoradiography, the sections were coated with a 1:2 dilution of Ilford K5 photoemulsion, air dried and exposed for one week in a light-safe box containing silica gel at 4°C. Slides were developed in D19 developer (Kodak), fixed in AGEFIX LIQUID (AGFA) and stained either with Giems or with haematoxylin/eosin.

**Results**

During early morphogenesis of the mouse (days 8.5 p.c. to 10.5 p.c.), in situ hybridisation analysis performed with antisense RNA probes revealed strong p53 hybridisation signals in all tissues of the embryo (Fig. 1A). As mouse development enters a phase of organogenesis and histogenesis, the p53 expression pattern became increasingly heterogeneous. Between days 12.5 and 18.5 p.c., most embryonic tissues were labelled more strongly by the antisense probe than by the sense probe that was used as negative control although p53 expression was clearly more pronounced in specific tissues (Fig. 1B–D).

**The placenta**

In the placenta of the day 10.5 p.c. mouse embryo, p53 expression was strongest in the chorionic villi (Fig. 1A). In contrast, the polyploid secondary giant cells of the trophoblast (syncytiotrophoblasts) did not reveal strong hybridisation signals (Fig. 1B). Within the maternal part of the placenta, the epithelial cells lining the peripheral venous plexus were clearly labelled (Fig. 1A).

**The yolk sac**

At day 10.5 p.c., the cells of the visceral yolk sac endoderm revealed moderate levels of p53 mRNA (Fig. 2). Expression was more prominent in small groups of hematopoetic precursor cells (blood-forming islands) located next to the endodermal wall of the visceral yolk sac (Fig. 1A). In contrast, the Reichert’s membrane was only weakly labelled (Fig. 2).

**The liver**

Between days 10.5 and 14.5 p.c. of mouse development, p53 was expressed at high levels in the fetal liver (Fig. 1B–D). Most cells of the developing liver were labelled although the intensity of the hybridisation signal was heterogeneously distributed. The endodermal prehepatocytes were apparently more strongly labelled than the mesodermal hematopoetic precursor cells within the blood-forming islands of the embryonic liver. A rapid decline of p53 expression occurred after day 14.5 p.c. and therefore coincided with the further differentiation of prehepatocytes, which is characterized by morphology changes of the cells from stellate to polygonal and with a concomitant expansion of the endoplasmic reticulum and glycogen storage (Medlock and Haar, 1983).

**The thymus**

The embryonic thymus contains a non-homogeneously distributed population of stromal cells and T-cell precursors at various stages of maturation. High levels of p53 expression were observed in all cells of the embryonic thymus at days 14.5 p.c. and 16.5 p.c. (Fig. 3A). However, at day 18.5 p.c., the p53 hybridisation signals became heterogeneously distributed (Fig. 3B). p53 gene expression was strongest in the large ovate reticulo-epithelial cells that form a compact stroma.

**The lung**

The characteristic budding pattern of the lung is the result of a continuous interaction of embryonic mesoderm and tracheal endoderm. As the trachea lengthens, it bifurcates at its caudal end to form two lung buds. These in turn continue to grow and branch, giving rise to the bronchial 'tree' of the lung. At day 12.5 p.c., p53 expression was strongest in tracheal cells. However, moderate levels of mRNA were also detected in the
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Fig. 1. Dark-field illuminations of p53 mRNA expression in mouse embryos. (A) Section through a day 10.5 p.c. decidua. mp, maternal placenta; ch, chorion. (B) Sagittal section through a day 12.5 p.c. embryo. li, liver; ne, neural epithelium; he, heart; op, olfactory pits. (C) Sagittal section through a day 14.5 p.c. embryo. lu, lung; tr, trachea; li, liver; in, intestine; te, telencephalon; me, mesencephalon. (D) Sagittal section through a day 16.5 p.c. embryo. bf, brown fat tissue; sg, salivary gland; lu, lung; li, liver; st, stomach; te, telencephalon.

early lung mesoderm. At day 14.5 p.c., high levels of p53 transcripts were found both in bronchial epithelia and in lung submucosa which is derived from mesodermal mesenchyme (Fig. 1C). At day 16.5 p.c., strongest p53 expression was discernible in the mesodermal cell population of the lung (Fig. 1D). At day 18.5 p.c., p53 expression in the lung appeared much weaker compared to earlier stages (not shown).

The kidney
During kidney development, reciprocal inductive interactions between the metanephric duct and the surrounding metanephrogenic tissue occur. The terminal portions of the metanephric duct induce the formation of metanephric tubules. Induction occurs in the upper cortex of the developing kidney. Regardless of the embryonic age, the cortical area consists predominantly of mesenchyme and epithelia at early stages of differentiation. Between day 14.5 and 18.5 p.c., strong p53 hybridisation signals subsequently became restricted to the uninduced mesenchyme and to the newly formed epithelia in the upper cortex (Fig. 4). The central parts of the kidney, which contain well-differentiated tubuli and glomeruli, were only weakly labelled by the p53 probe (Fig. 4).

The intestine
The intestine of the young embryo has a multilayered endodermal epithelium. At day 14.5 p.c., all cells of the stratified intestinal epithelium were strongly labelled. By day 15 p.c. of mouse development, aggregates of mesodermal cells begin to invade the stratified intestinal epithelium, which forms small secondary lumina beneath its surface. Coalescence of secondary lumina and continued mesodermal upgrowth result in the formation of the fingerlike intestinal villi, which greatly
increase the absorptive surface of the intestine. At the base of the villi are tubular invaginations called intestinal crypts.

By day 16.5 p.c. of mouse development, the epithelial cells within the intestinal crypts were apparently more strongly labelled than cells covering the villi (Fig. 5). The mesodermal cells forming the submucosa revealed only low levels of p53 mRNA.

The teeth

Tooth development is the result of reciprocal inductive interactions of two tissues of different origin. Neural crest cells induce the oral epithelium to form the enamel epithelium which in turn induces the neural crest cells to build up the dental papillae. The enamel epithelium gives rise to the enamel-forming cells of the enamel organ while the cells of the dental papillae later differentiate to tooth dentine.

At day 16.5 p.c., p53 expression was strongest in the outer layer of ameloblasts. The cells of the inner enamel epithelium were only weakly labelled. A strong hybridisation signal was also visible within the dental papillae (Fig. 6).

The salivary glands

The growth and morphogenesis of the salivary glands is based on continued interactions between the salivary epithelium and the associated mesenchyme.

At days 14.5 and 16.5 p.c., the developing salivary epithelium revealed much higher levels of p53 expression than the surrounding mesenchyme (Fig. 1D).
At day 18.5 p.c., p53 expression in the salivary gland epithelium was greatly reduced.

The hair follicles
In mouse development, formation of hair follicles is visible as early as day 13 p.c. when the vibrissae papillae are still invaginated and ectoderm is proliferating in the external nares. At days 14.5 p.c. and 16.5 p.c., a strong p53 expression was discernible in the ectodermal epithelia of the external root sheath (Figs 1D and 9). In contrast, the primordia of the dermal sheath showed only low levels of p53 expression.

Fig. 4. Expression of p53 mRNA in the kidney at day 18.5 p.c. Left: bright-field illumination; right: dark-field illumination. me, medulla; co, cortex.

Fig. 5. Expression of p53 mRNA in the intestine at day 18.5 p.c. Left: bright-field illumination; right: dark-field illumination.

Fig. 6. Expression of p53 mRNA in the tooth bud at day 16.5 p.c. Left: bright-field illumination; right: dark-field illumination. dp, dental papilla; e, enamelepipethelium.
At day 16.5 p.c. when body hair formation in the skin is first recognized, high levels of p53 expression were clearly visible in the epidermal hair bulb that partially surrounds the dermal papilla like an inverted cap. This pattern of expression was still visible at day 18.5 p.c. (Fig. 7).

The brain
At early stages of nervous tissue development (days 10.5 p.c. and 12.5 p.c.), all neuroepithelial cells were labelled strongly (Fig. 1B). As the neuroepithelium matures, the innermost ventricular layer contains cells that are still in the mitotic cycle. Some of the daughter cells generated in the neuroepithelium lose their ability to undergo mitosis and migrate towards the outer wall of the neuroepithelium. These neuroblasts later differentiate into individual neurons. At day 16.5 p.c., p53 expression was strongest in proliferating neuroepithelial cells surrounding the ventricles of the cerebellum, the telencephalon and the mesencephalon. However, moderate p53 expression was also visible in the outermost mantle zone consisting of postmitotic cells (Fig. 1C,D). At day 18.5 p.c., the outer neuroblast cell layer of non-dividing cells and the ventricular layer showed equal levels of p53 mRNA (Fig. 8).

The nasal chamber
By day 12.5 p.c., p53 expression was clearly detectable in the olfactory pits (Fig. 1B). At day 16.5 p.c., Jacobson’s organ was strongly labelled. A moderate level of p53 mRNA expression was also detected in the olfactory epithelia (Fig. 9).

The brown adipose tissue
The brown adipose tissue is derived from rapidly proliferating fibroblast-like cells mid-dorsally between the muscles of the dorsal thorax. These pre-adipocytes are arranged in clusters and differ in morphology from the surrounding dermal tissue. The developing brown adipose tissue rapidly increases in size until day 18 p.c. and then differentiates to mature adipocytes which build up a fat cushion in the neck region.

At day 16.5 p.c., high levels of p53 mRNA were found in the pre-adipocytes (Fig. 1D). However, at later stages when fibroblast-like fat cell precursors...
differentiate to mature brown adipocytes, p53 expression clearly declined.

**Discussion**

The results of the present study support the general findings of earlier reports on p53 gene expression during embryogenesis. Mora et al. (1980) detected p53 protein in primary cells of day 10 to day 14 mouse embryos. These authors also found detectable amounts of p53 protein in day 13 mouse embryo liver but not in liver of day 15 mouse embryos which coincides with the rapid decline of p53 mRNA that we observed in the liver after day 14.5 p.c. The levels of p53 mRNA in fetuses at day 9 to day 11 of gestation were similar to those seen in some tumors and a marked decrease was observed from day 11 onwards (Rogel et al. 1985). Louis et al. (1988) have shown that the decline in p53 protein is equivalent to the decline in the p53 mRNA level and that down-regulation of p53 mRNA is post-transcriptionally regulated. Furthermore, down-regulation of p53 mRNA during embryonic development has been observed during chicken and mouse embryogenesis, thereby implying that p53 may have an essential role in embryonal differentiation.

However, no detailed analysis of p53 gene expression during embryogenesis has been published to date that gives a more profound insight into the role that p53 plays during development.

In this report, we have described the first in situ study of p53 gene expression during mouse embryogenesis. We have demonstrated that p53 is strongly expressed in all tissues of the early embryo. However, as development proceeds towards a phase of organogenesis which is characterized by complex differentiation events, dynamic and tissue-specific p53 expression patterns were observed. At these stages of development, high levels of p53 transcripts were found predominantly during early differentiation events. In tissues undergoing terminal differentiation, p53 mRNA expression declined.

Reports from several experiments using different approaches suggest that p53 may play a central role in regulation of cell growth. Microinjection of p53 antibodies into the cell nucleus blocked the entry into the S-phase (Mercer et al. 1984) and, in other experiments, expression vectors producing antisense p53 mRNA markedly impaired the growth of mouse cells in vitro (Shohat et al. 1987). These data suggest that p53 is essential for cell-cycle related growth processes and this could also explain the high levels of p53 mRNA that we observed in the rapidly proliferating undifferentiated cells of the early embryo.

Constitutive expression of wild-type p53 has however, been shown to be antiproliferative in SV40-transformed hamster cells, whereas mutant forms of p53 had no effect (Mercer et al. 1990) and similar results have been reported in cell lines derived from colon cancers (Baker et al. 1990). Conditional expression of wild-type p53 in a cell line derived from a human glioblastoma tumor blocked cell cycle progression (Mercer et al. 1990). Recently, it was reported that growth suppression induced by wild-type p53 protein is accompanied by selective down-regulation of proliferating cell nuclear antigen expression (Mercer et al. 1991), a protein that has been identified as a cofactor for DNA polymerase δ, an important component of cellular DNA replication.

These contrasting results suggest that the effect of wild-type p53 on cell growth may be dependent on the cell type examined. However, since recent studies have shown that human p53 protein is differentially phosphorylated (Bischoff et al. 1990), it is tempting to speculate that post-translational modifications may contribute to these differing effects of p53 protein on cell proliferation.

It is interesting to note that, at later stages of mouse embryonic development, the levels of p53 mRNA expression did not correlate with cell proliferation. This was most prominent in the fetal brain where postmitotic, but not yet terminally differentiated cells showed clearly detectable levels of p53 mRNA.
What is the biological significance of the complex p53 expression pattern that is observed during late organogenesis? Our overall hypothesis is that p53 is involved in the regulation of cellular differentiation processes and this view is supported by the findings of several in vitro studies. During the induced terminal differentiation of murine erythroleukemia (MEL) cells, down-regulation of p53 protein (Shen et al. 1983) and mRNA (Khochbin et al. 1988) was observed. Similarly in mouse F9 teratocarcinoma cells, the levels of p53 protein (Reich et al. 1983) decreased following retinoic-acid-induced differentiation, and the down-regulation seemingly occurring at the post-transcriptional level (Dony et al. 1985). Furthermore, the decline of p53 protein in F9 mouse teratocarcinoma cells correlated with cellular differentiation and not with proliferation (Chandrasekaran et al. 1982).

The strong p53 hybridisation signals that we found confined to specific differentiation stages of certain tissues suggest that p53 is involved in cell maturation. Up-regulation of p53 gene expression in proto-differentiated cells may be a prerequisite to inhibit cell cycle progression and to induce terminal differentiation of certain tissues.

Various human and murine tumor cell lines have been shown not to contain a functional p53 gene as a result of gross deletions or insertions in either allele (Wolf et al. 1984a,b; Mowat et al. 1985; Ben-David et al. 1988). More recently, germ line p53 mutations were identified in a familial cancer syndrome (Srivastava et al. 1990; Malkin et al. 1990) suggesting that the loss of normal p53 function may be conducive to tumorigenesis and implying that normal p53 may function as a tumor suppressor or 'anti-oncogene'. If p53 does play a pivotal role in the regulation of cell differentiation events, loss of p53 function might prevent terminal differentiation and maintain proliferation in this way. Sites of strong p53 expression in development may therefore represent those tissues and differentiation stages that are susceptible for transformation by loss of p53 function.

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References


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