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p53 in embryonic development: maintaining a fine balance

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Abstract. In addition to its role as a tumour suppressor and cell-cycle checkpoint control protein, p53 has been implicated as an important protein in embryonic development. Despite the viability of most *p53* null mice, evidence has accumulated that p53 may regulate differentiation and the response of embryonic cells to diverse environmental stresses. Moreover, it appears that maintenance of a fine balance of p53 protein levels within embryonic cells is important for optimal development. Inappropriate overexpression or underexpression of p53 can lead to embryonic lethality or increased risk of malformations. The p53 protein may utilize multiple functional activities in its regulation of developmental processes.

Key words. p53; differentiation; embryonic development; tumour suppressor; teratological suppressor; apoptosis; knockout mice; cell cycle.

Introduction

Experiments with 'knockout' mice in the last several years have revealed that most of the prototypical human tumour suppressor genes are essential for embryonic development [1]. Inactivation of both germ line alleles of a tumour suppressor usually results in embryonic lethality either in early or midgestation. Formation of critical organ structures or even completion of early proliferation and differentiation processes may be impossible in the absence of a particular tumour suppressor gene. One of the notable exceptions to this pattern has been the p53 tumour suppressor. Most mice missing both functional copies of *p53* exhibit normal prenatal and postnatal development [2]. The developmental viability of *p53* null mice was initially surprising, given the importance of p53 as a known cell-cycle checkpoint control protein and its high levels of expression in early phases of embryonic development [3–5]. Moreover, significant evidence had accumulated that p53 could induce differentiation in a number of in vitro model systems [6, 7]. However, since the findings of apparent p53 dispensability in development, the pendulum has again swung back towards the position that p53 does play an important role in embryonic development. Overexpression or underexpression of wild-type p53 can clearly have detrimental effects on development. Some recent data have indicated that p53 may play a key role in protecting embryos from teratogens and diverse environmental stresses [7–9]. The known or suspected activities of p53 in modulating embryonic development will be the subject of this review. The primary emphasis of this review will be on whole animal model studies, with secondary references to those cell culture studies that impact on our understanding of p53 in organismal development.

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Expression and functional activity of p53 in the developing embryo

Since the role of p53 in regulating the cell cycle, protecting the genome from DNA damage and in induction of apoptosis is discussed in other reviews in this issue, we will not address these topics in any detail, except as they relate to development. Suffice it to say that all of these p53-associated functions are likely to be involved in at

least some stage or in some cells of the developing embryo.

The first studies to implicate p53 in development were those examining *p53* messenger RNA (mRNA) and protein expression in developing mouse embryos [3–5]. Both mRNA and protein are expressed at high levels in embryos up to midgestation [11 days post-coitum (p.c.)], and then expression levels rapidly decrease. In situ hybridization on fixed embryo sections revealed that all cells expressed *p53* mRNA until 10.5 days p.c., but at later organogenesis stages only specific differentiating tissues showed high levels of expression. Terminally differentiated tissues exhibited very low levels of *p53* mRNA [5]. Chicken embryos showed similar progressive downregulation of p53 mRNA and protein in the second half of their embryonic development [4]. Thus, in developing embryos, there appeared to be a very good correlation between p53 levels and the fraction of embryonic cells undergoing proliferation.

The high level of p53 expression in early embryos suggests a functional role for the protein during these proliferative stages. However, recent studies using transgenic models indicate that p53 protein may be relatively inactive during embryogenesis, at least with respect to some functions. Three groups have developed *lacZ* transgenic mice in which a bacterial *lacZ* marker gene was driven by a promoter with an artificial or natural *p53* DNA response element, making the *lacZ* gene responsive to upregulation by activated p53 [10–12]. The *lacZ* gene (encoding β-galactosidase) is a particularly useful marker because its expression is readily detectable in fixed tissue sections following incubation with the chromogenic β-galactosidase substrate X-gal (cells expressing β-galactosidase stain blue). Surprisingly, despite high levels of p53 protein throughout early embryos, high levels of β-galactosidase activity were observed only in the developing nervous system even into the late stages of embryogenesis. These results suggest a posttranslational level of control on the transcriptional activity of p53. When the *lacZ*-containing embryos were subjected to ionizing radiation, many early embryonic tissues stained blue, though staining became more restricted with increasing age of the embryo. Overall, the expression of *lacZ* following radiation correlated well spacially and temporally with expression of p53 mRNA and protein and induction of apoptosis, indicating that most p53 protein in the embryo was normally in a latent state but fully capable of being functionally activated under the appropriate stressor.

That p53 protein may be latent in its functional activity was further corroborated by Lutzker and Levine [13] in their analysis of p53 in undifferentiated teratocarcinoma cell lines. Teratocarcinoma cell lines and embryonic stem cells express high levels of p53 protein, but it was demonstrated that endogenous and exogenously added p53 transcriptional targets were not upregulated in the murine teratocarcinoma lines. However, exposure of the teratocarcinoma lines to DNA-damaging drugs extended the p53 protein half-life and resulted in transcriptional activation of p53 target genes and apoptosis (programmed cell death) of the treated cells. Interestingly, induction of differentiation by retinoic acid decreased p53 mRNA and protein levels, yet resulted in increased transactivation of p53 target genes in a p53-specific manner. These paradoxical results demonstrate that p53 protein levels and functional activity do not always correlate well and should induce caution in making any conclusions about p53 activity based solely on quantitative levels of p53 mRNA or protein.

p53 and cell differentiation

Currently, there is an extensive body of literature linking p53 to differentiation processes (see Almog and Rotter [6] for review). A number of in vitro and in vivo assays have clearly demonstrated that adding exogenous wild-type p53 to undifferentiated cells can result in progression from an undifferentiated to a more differentiated state. A prototype series of experiments was described by Rotter and colleagues in their work with the undifferentiated early pre-B cell line L12, which lacks wild-type p53 [14, 15]. Introduction of wild-type p53 into these cells induces the expression of the μ immunoglobulin heavy chain and the B-cell surface marker B220, as well as an increase in the fraction of cells in G0/G1. Irradiation of another pre-B cell line containing wild-type p53 induced differentiation (which was inhibited by mutant p53), as evidenced by an increase in κ light chain mRNA expression, suggesting that radiation-induced p53 activation was the differentiation inducer [16]. Increased levels of wild-type p53 in diverse haematopoietic cell types, including those of myeloid, granulocyte or erythroid lineages, were also correlated with various lineage-specific differentiation markers, indicating induction of differentiation by p53 [6]. Other non-haematopoietic cell types have also been made to differentiate by addition of exogenous p53. Myogenic cells, osteogenic cells, keratinocytes, oligodendrocytes, neurons and thyroid cells were induced to differentiate by addition of wild-type p53 or could have their normal differentiation pathways blocked by addition of dominant negative mutant versions of p53 [6].

How can the apparently ubiquitous involvement of p53 in various differentiation pathways be reconciled with the normal differentiation seen in *p53* null mice? p53 appears to be engaged in differentiation, yet is obviously not an essential requirement for it. One argument could be that differentiation depends on the interactions of many regulatory molecules, and that the loss of one of these molecules is not catastrophic due to the flexibility or redundancy built into this complex process. The recent isolation of novel p53 family members provides support for the idea of p53 redundancy and provides a mechanism by which cellular processes normally dependent on p53 could be compensated for [17, 18]. Alternatively, there might be multiple initiators or pathways to differentiation. An example of this scenario was observed in the analysis of *SCID* mice lacking the ability to resolve the immunoglobulin gene rearrangements initiated during T-cell differentiation. The T cells of the *SCID* mouse are blocked at the undifferentiated CD4− CD8− precursor stage. However, ionizing radiation was found to stimulate V(D)J rearrangements in T-cell receptor genes and to result in differentiation of CD4− CD8− T-cell precursors to more mature CD4+ CD8+ forms [19, 20]. We were able to show that this radiation-induced T-cell differentiation and V(D)J rearrangement was dependent on the presence of p53 [21]. Interestingly, progression of *SCID* T cells to the more mature CD4+ CD8+ stage could also be induced by anti-CD3 antibody, independent of p53 status, suggesting that there are alternate routes of p53-dependent and -independent differentiation in this model.

Another possibility is that the primary role of p53 is not to initiate differentiation, but to provide the cellular conditions necessary for the functioning of the normal differentiation factors. In this type of scenario, activation or overexpression of p53 may induce a cell-cycle arrest, reducing the activities of factors which spur cell-cycle progression and favouring the actions of factors which might induce differentiation. In many of the models cited above, induction of p53-mediated differentiation was accompanied by cell-cycle arrest or apoptosis. Thus, according to this model, molecules other than p53 that initiate a cell-cycle arrest might also induce differentiation, consistent with the dispensability of p53 in organismal differentiation.

Embryonic development in the *p53* null mouse

One of the most direct ways to understand the role of a gene in development is to inactivate it in the germ line of an organism. Gene-targeting techniques in embryonic stem cells have now made this approach possible for mammalian organisms, particularly the mouse. We and others have successfully generated mice with germline *p53* null mutations [2, 22–25]. When our heterozygous *p53* mice were intercrossed, 23% of the offspring were null for *p53*, roughly the expected Mendelian ratio. The *p53* null mice were developmentally normal in every measurable way, except that they were predisposed to early tumour development (primarily lymphomas and sarcomas). Other laboratories also reported viable *p53* null mice, utilizing targeting constructs which deleted larger portions of the *p53* gene, confirming that the viability of our *p53*−/− mice was not simply due to incomplete gene inactivation [22, 23, 25]. However, shortly after our initial report, Rotter and colleagues [26] described a defect in spermatogenesis in some *p53*-deficient mice. *p53*−/− males of 129/Sv background showed a high frequency of multinucleated giant cells within the testicular seminiferous tubules, believed to be a result of inability to complete meiosis. We and others have observed that the *p53* null females and males have a lower fertility than their wild-type and *p53*+/− littermates [27, 28].

In contrast to the 23% null offspring resulting from our heterozygote crosses, Jacks and colleagues observed a 20% null frequency [29], and Clarke and colleagues reported a deficiency in numbers of *p53* null females [28], suggesting the possibility of an embryonic defect of incomplete penetrance in the null animals. Further examination of embryos by these groups revealed that a substantial fraction (8–23%) of the female *p53* null embryos exhibited a neural tube closure defect called exencephaly [28, 29]. Exencephaly consisted of an outgrowth of neural tissue usually confined to the region of the fore- and midbrain, but sometimes also affecting the hindbrain. The neural outgrowth prevented normal cranium formation, so that the brain appeared to be on top of the skull. The Jacks lab reported the exencephalic defect as the primary defect, whereas the Clarke group described additional abnormalities in the null embryos, including upper incisor fusion, ocular abnormalities and polydactyly of the hindlimbs [28, 29]. Moreover, irradiation of null embryos resulted in dramatic increases in the frequency of exencephaly [28].

The reports on exencephaly in the *p53*-deficient mice raise a number of questions. For example, why was exencephaly observed? Why does incomplete penetrance occur? Why are females affected rather than males? What is responsible for the variability in penetrance observed by different groups in different strains? The observation of exencephaly is actually consistent with the fact that p53 expression levels and activity in the *lacZ* transgenic mice described earlier was highest in the developing nervous system [10–12]. Therefore, this particular organ system might be exp-

ected to be the most severely affected in the absence of p53, perhaps due to a failure to cell-cycle arrest or a failure of induction of apoptosis in neuronal precursor cells at critical times in neural tube development. It has been shown from studies of neural tube closure that neural folds may develop as consequence of local changes in cell turnover, and these turnover rates could be affected by the absence of p53. The incomplete penetrance might be due to the interaction of genetic and environmental factors. Sah et al. [29] demonstrated that 129/Sv *p53*−/− null embryos had a higher rate of exencephaly than null embryos of mixed 129/Sv-C57BL/6 background, suggesting that strain-specific modifier genes may influence the degree of penetrance. Radiation increases exencephaly rates in null mice [28], and we have observed that lowering folic acid concentrations in the mouse chow can augment the frequency of *p53*null exencephaly (A. Sands, L. Donehower, A. Bradley, unpublished data). The fact that our regular mouse chow is higher in folic acid than most commercial mouse chows may partially explain the lower incidence of exencephaly in our colony compared with the Jacks and Clarke colonies. The female bias in exencephaly is not easily explainable, but Armstrong and colleagues [28] have noted that a female-biased sex distortion has been reported historically for both humans and mice. Perhaps the absence of p53 somehow exacerbates this distortion.

The discovery that an absence of p53 activity can lead to a fatal defect in a significant fraction of embryos clearly implicates p53 as an important factor in development. Elucidating the role of p53 in neural tube closure in mice may lead to a better understanding of this protein both as a cell-cycle regulator as well as a developmental regulator. In addition, the *p53* null embryos may represent a useful model for the study of the aetiology of embryonic neural tube defects.

p53 and development in other animal models

The absence of p53 is clearly compatible with development in some mice. However, other animal models have been generated in which alterations in p53 expression and structure have been shown to be deleterious to development. The first transgenic animal models for p53 were generated by Bernstein and colleagues [30], who generated mice containing mutant transgene forms of murine genomic *p53*. Developmental abnormalities for two of the mutant transgenic mice were not reported, but in a subsequent study we found that in crosses of one of the mutant transgenic mice to non-transgenic mice there was a significant (2:1) bias against transgenic offspring [31]. However, even in the absence of endogenous wild-type p53, the overexpressed mutant transgene did not appear to impair development [31]. Not surprisingly, the transgenic mice were susceptible to spontaneous lymphomas, sarcomas and carcinomas [30, 31]. Another transgenic mouse with an internal deletion in genomic *p53* (deleting exon 2 and flanking intron sequences and generating a truncated protein of 44 kDa) failed to generate any founder transgenic mice despite injection of over 1800 fertilized eggs [30]. Moreover, attempts to generate transgenic mice containing overexpressed copies of wild-type *p53* have failed (A. Bernstein, J. Butel, personal communications). Thus, while absence of p53 may be compatible with development, greater than normal physiological levels of wild-type and some mutant forms of p53 are likely to be injurious to normal embryonic development.

Ectopic expression of mutant and wild-type forms of p53 directed to specific tissue compartments have also revealed some interesting developmental phenomena. Godley and colleagues [32] have shown that wild-type *p53* constructs driven by a mouse mammary tumour virus (MMTV) promoter targeting expression to the mammary gland resulted not in mammary gland abnormalities but in derangement of kidney development. These transgenic mice had kidneys half their normal size due to defective differentiation of the ureteric bud, accompanied by high rates of apoptosis in the metanephric mesenchymal cells, producing inefficient conversion to renal epithelial cells. The end result was defective kidney function, kidney disease and death.

In a related approach, Westphal and colleagues [33] have expressed wild-type p53 in the developing lens of transgenic mice and shown that these mice developed microphthalmia. Apoptotic cells were observed, and these had failed to undergo proper differentiation. Expression in the mammary gland of a mutant murine *p53* gene (codon 172 Arg→Leu) behind an MMTV promoter by Rosen and colleagues [34] led to female mice with an impaired ability to lactate due to a failure in normal lobuloalveolar development in the mammary gland. Further analysis revealed that this particular mutant form of *p53* (analogous to mutations identified in some human breast cancers) actually behaved like a wild-type version of *p53*. It transactivated normal p53 targets, mediated radiation-induced apoptosis and even made mice more resistant to carcinogen-induced mammary carcinomas than normal nontransgenic mice [34, 35].

The effects of p53 on *Xenopus laevis* development have also been examined. *p*53 transcription is activated during early oogenesis, and zygotic transcription initiates in the embryo after the midblastula transition. Unlike mice and chickens, where *p*53 levels tend to decline after midgestation, the levels of *p53* transcripts appear to remain relatively high throughout all the tissues during

development [36]. When wild-type *p53* transcripts were microinjected into two and four cell embryos, lethal embryonic defects until and during gastrulation were observed. In some cases normal cell cleavages were completely arrested or greatly retarded. A variety of developmental defects were observed, consistent with inappropriate cell-cycle inhibition by overexpressed p53 during development.

In the opposite type of approach, when dominant negative mutant p53 or Xdm2 (the *X. laevis* homologue of *mdm2*, a potent inhibitor of p53 activity) were injected into *X. laevis* early embryos, the inactivation of p53 function by these two p53 inhibitors prevented *Xenopus* early blastomere differentiation and resulted in the formation of large cellular masses resembling tumours [37]. For example, dominant negative *p53* transcripts injected into blastomere cells fated to give rise to brain and spinal cord resulted in a large tumour embedded within the brain region which expressed no neural differentiation markers. Interestingly, when the same mutant *p53* RNA was injected into all cells at the four-cell stage, no abnormal cell-cycle perturbations were observed until the midblastula transition [37]. Since early embryos up to the midblastula transition do not have cells with G1 or G2 phases, this result suggests that the primary cell-cycle inhibitory effects were either in G1 or G2.

The inactivation of wild-type p53 activity clearly prevented normal development in the *Xenopus* embryo, in contrast to the lack of effect of p53 inactivation in the murine embryo (excepting the occasional exencephaly). Although the possibility that the mutant *p53* and *Xdm2* genes used in these studies had a gain of function activity that inhibited differentiation and development cannot be ruled out, a more likely explanation is that mice have genetic redundancies that frogs do not have. These mammalian genetic redundancies have been well documented for other genes such as *MyoD* and *Myf-5*, which when mutated alone do not affect muscle development, but mutated together do so [38]. Thus, the *Xenopus* results support the idea that p53 does play a role in differentiation and development, but one that is potentially masked in mammalian systems by other compensating genes.

p53 and development in the context of other genetic deficiencies

As a transcriptional factor, p53 has been demonstrated to regulate a number of genes involved in cell-cycle control, apoptosis, and checkpoint function [39–41]. Moreover, p53 appears to bind an amazingly large number of proteins involved in growth control, DNA repair and transcriptional regulation. Given the many molecular functions and interactions mediated by p53, it is perhaps not surprising that in the last few years it has been shown to influence developmental processes in the context of other genetically deficient knockout mice. Usually, this interaction takes the form of partial or complete rescue of an embryonic lethal phenotype following crosses of *p53*-deficient mice to a mouse deficient in a growth regulatory or DNA repair-related gene. Such crosses have provided important new insights into the interactions of *p53* and other genes during differentiation, development, DNA repair and growth control.

The most dramatic example of phenotype rescue by *p53*-deficiency was observed when *mdm2*-deficient mice were crossed to *p53*-deficient mice [42, 43]. The *mdm2* gene encodes a protein which directly binds to p53 and inhibits its normal transcriptional activation and tumour suppressor functions at least in part through p53 degradation [44, 45]. Moreover, p53 transactivates *mdm2* gene expression, providing another level of interaction between the two genes. We and the Lozano laboratory found that *mdm2* nullizygosity resulted in early embryonic lethality at roughly 6.5 days gestation [42, 43]. *Mdm2* null embryos at 6.5 days were half their normal size, and showed profound disorganization of normal embryonic structures. However, after crosses of *p53*-deficient mice to *mdm2* heterozygous mice, double null *p*53 and *mdm2* mice were found to be completely viable with no obvious developmental abnormalities. This surprising rescue of the embryonic lethality of *mdm2* null mice by *p53* nullizygosity indicated that the primary role of *mdm2* in development was to inactivate p53 activity. It is known that the time of the developmental block in the *mdm2* null mice coincides with a sudden increase in cell-cycle rate that occurs at day 5.5–6.0 of development [46]. Thus, we hypothesized that the role of mdm2 was to inactivate p53 and prevent inhibition of cell-cycle progression during this accelerated cycling period. This idea was consistent with our demonstration of high levels of *mdm2* and *p53* mRNA during the critical 5.5–6.5-day developmental window [42]. Interestingly, rescue of the *mdm2* null embryonic lethality could not be achieved by substituting $p21^{WAF1/CIP1}$ nullizygosity for *p53* nullizygosity, suggesting that abrogation of the G1 arrest checkpoint function of p53 was not involved in the rescue [47].

Another example of rescue by *p53*-deficiency was illustrated in the *Rad51-p53* knockout mouse crosses [48]. Mammalian homologues of yeast *Rad51* are believed to repair double-stranded DNA strand breaks by recombination [49]. Rad51 protein has recently been found to bind p53 [50, 51]. It has been hypothesized that one role of p53 binding to Rad51 is to inhibit promiscuous recombination. *Rad51* null mice are arrested in development at the 5.5-day early egg cylinder stage [48]. The

cells from null embryos exhibited reduced proliferation, loss of chromosomes and hypersensitivity to ionizing radiation, consistent with a failure to repair DNA double-strand breaks. Since p53 induces a G1 arrest in response to DNA strand breaks, intercrossing of *Rad51* and *p53* knockout mice was a logical experiment. In fact, *p53* nullizygosity partially rescued the *Rad51* null embryos by extending their development to the 8.5-day stage compared to 5.5 days in the presence of p53 [48]. Thus, it appears that the *Rad51*−/− embryonic cells had sustained levels of damage low enough to continue dividing in the absence of p53 cell-cycle checkpoint control. The presence of p53 may activate a checkpoint which arrests the *Rad51*−/− embryonic cells early in development and results in lethality. Presumably, in the absence of p53, the double-mutant embryos died later from an accumulation of DNA damage too extensive to permit DNA replication or mitosis.

Nullizygous embryos deficient in the *Brca1* and *Brca2* tumour suppressors have also been partially rescued by the absence of p53 [52, 53]. Inherited mutations in the human *BRCA1* and *BRCA2* genes have been shown to be responsible for an elevated predisposition to breast cancer, and the encoded proteins have been implicated in recombinational repair, in part through their interactions with *Rad51* [54, 55]. *Brca1* and *Brca2* null embryos show early developmental blocks at days 5–6 and 7.5–8.5 of gestation, respectively [55, 56]. Embryos lacking either of the two genes showed reduced levels of proliferation, reminiscent of the *Rad51* null embryos. Introduction of *p53* nullizygosity into the *Brca1* and *Brca2* null embryos resulted in an extended embryo survival of 1 to 2 days [52, 53]. Again, these data show striking similarities to the *Rad51-p53* crosses and suggest that Rad51, Brca1 and Brca2 are all involved in the same recombinatory repair pathways and may interact in a similar way with p53.

Tissue-specific partial rescue by *p53* deficiency was observed in *ATM*-null mice, the genetic model for the human disorder ataxia telangiectasia, characterized by various neurological malfunctions, infertility and increased tumour susceptibility [57]. The *ATM* null males have severely disrupted gametogenesis in the earliest stages of prophase I. Introduction of two *p53* (or *p21*) null alleles into the *ATM*−/− background resulted in the further progression of gametogenesis to the pachytene stage of prophase I [57]. Defective T-cell differentiation and V(D)J recombination in *SCID* mice (defective for the DNA-dependent protein kinase) has also been partially rescued by *p53* deficiency [21, 58, 59].

p53 as a teratological suppressor

The demonstration of radiation-inducible transcriptionally active p53 in early and midgestation embryos in the *lacZ* transgenic models clearly indicates that the p53-mediated DNA damage response is intact in development. If the ability to respond to DNA damage is intact, then it is likely that other p53-associated responses to diverse environmental stresses are present in the embryo. This obviously has important implications for the role of p53 in responding to teratogens. Confirmation of p53 as a teratological suppressor has been provided by two studies using chemical and physical teratogens. Nicol and colleagues [8] treated pregnant *p53*+/− and *p53*+/+ females (mated to *p53*+/− males) at gestation day 10 (a time of high teratogen susceptibility) with the teratogen and carcinogen benzo[a]pyrene. They showed that *p53*+/− mothers had twice as many foetal resorptions and fourfold more overall embryo lethality than *p53*+/+ mothers. Moreover, regardless of maternal genotype, the *p53* genotype of the embryos was particularly important. In utero death induced by benzo[a]pyrene occurred in 26% of *p53*+/− and 36% of *p53*−/− embryos compared with deaths in 10% of *p53*+/+ embryos. The enhanced susceptibility of *p53*+/− embryos was particularly striking, indicating that even a reduction in p53 dosage is sufficient to significantly increase teratogen effects. This finding may have important implications for Li-Fraumeni syndrome family members, who are predisposed to early cancer development due to a germ line mutation in one of their *p53* alleles. The affected foetuses in such families may be more susceptible to the effects of environmental teratogens.

In a related type of teratogenesis experiment, Norimura and colleagues [9] treated 9.5-day-old *p53*+/+ and *p53*−/− foetuses with ionizing radiation. *p53*−/− embryos irradiated with 2 Gy exhibited a 70% incidence of developmental abnormalities and a 7% incidence of deaths, whereas *p53*+/+ embryos had a 20% incidence of anomalies and a 60% incidence of deaths. The presence of p53 in the irradiated embryos was also correlated with high levels of apoptosis not observed in the *p53*−/− embryos. This remarkable p53-dependent inversion of phenotype provides strong support for the idea that p53 efficiently aborts embryonic cells with teratogen-induced DNA damage [7]. If too many cells of the embryo die through apoptosis, then the entire embryo will die. Conversely, lack of p53 in the embryo presumably results in continued cell-cycle progression despite DNA damage, much less apoptosis and a higher proportion of embryos with developmental anomalies.

A third study related to teratogen effects actually showed a paradoxical protective effect in the absence of p53. Wubah and colleagues [60] showed that treatment of 8-day-old embryos with the genotoxic agent 2-chloro-2-deoxyadenosine resulted in 73% incidence of eye defects in *p53*+/+ embryos, but only 2% for *p53*−/− embryos. Again, the heterozygotes showed a intermediate incidence of 52% eye abnormalities. The eye

defects were correlated with induction of apoptosis in the developing head folds, indicating that the induction of p53-dependent apoptosis in response to DNA damage is not always phenotypically beneficial if the effects are relatively localized.

Despite the results of Wubah and colleagues [60], it is probably safe to conclude that an important role of p53 is as a teratological suppressor. In fact, Hall and Lane, in a recent review, have argued that this particular p53 'guardian of the babies' function may be of even greater significance than its role as a tumour suppressor [7]. They postulate that p53 may have evolved in the more complex organisms (it has not yet been found in lower organisms such as *Drosophila*) to provide a more flexible multicellular adaptive response to a variety of environmental stresses. This adaptive p53-mediated response would facilitate the evolution of greater developmental complexity. It would certainly make good evolutionary sense to more efficiently abort foetuses with even small increases in DNA damage if the failure to do so is an offspring with reduced reproductive fitness. The importance of p53 in preserving reproductive fitness even in the absence of obvious environmental stress is underscored by the reduced fertility of both *p53*−/− males and females and the exencephaly incidence in the embryos [27–29].

Conclusions, speculations and models

Despite the confounding viability of the *p53* null mice, the importance of p53 in development has been unequivocally demonstrated in the last several years. Indeed, it is apparent that not only is p53 significant in developmental processes, but the relative levels of p53 in the cell are also highly important. Deviation from normal wild-type p53 levels in either direction can have serious detrimental consequences. Generalized overexpression of wild-type p53 is clearly incompatible with development as introduction of additional wild type, and some mutant forms of p53 in transgenic mice fail to generate viable offspring [30]. Moreover, if overexpression of wild-type p53 is localized, significant organ malformations result [32–34]. Even physiological levels of wild-type p53 expression can be highly detrimental to development if not counteracted by appropriate expression of *mdm2*, as is observed in the early embryonic lethality of mdm2 null mice [42, 43]. This embryonic lethality is almost certainly due to an inappropriate abundance of uncomplexed p53 during a critical developmental window.

In many instances, reduction in p53 levels can be deleterious in development. The 2.6-fold increase in foetal lethality in the benzo[a]pyrene-treated *p53*+/− embryos over *p53*+/+ embryos testifies to the effects of even a 50% reduction in p53 levels following teratogen insult [60]. The significance of this dosage effect has also been shown by Oren and colleagues following radiation of the p53-responsive *lacZ* transgenic mice, which are heterozygous for endogenous *p53* [11]. The *p53* heterozygote *lacZ* mice showed a greatly reduced transcriptional response compared with *p53*+/+ *lacZ* mice which was only marginally higher than that of the nonresponding *p53* null *lacZ* mice. Of course, the complete absence of p53 is profoundly deleterious, resulting in reduced fertility, high incidence of exencephaly, and high susceptibility to the effects of environmental stresses [27–29]. Thus, the maintenance of a fine balance of cellular p53 levels appears to be a critical aspect for development and for subsequent protection from tumours.

Given the multifunctional nature of p53, a key question arises with regard to the nature of p53 activities which are essential for development. The teratogenesis experiments have clearly shown that the p53-dependent DNA damage response pathways are highly active in the developing embryo [8, 9, 60]. The transcriptional activation and apoptotic responses are correspondingly intact throughout the embryo, at least through midgestation [10–12]. However, it was noted that p53-related transcriptional activities were relatively quiescent in unstressed mice. There is compelling evidence that dividing cells, particularly early embryonic cells, can maintain p53 in a relatively latent state in the absence of mutational or physiological stressors [13, 61]. If the early embryo does maintain a latent form of p53, why are null *mdm2* embryos blocked in development, whereas double null *mdm2*, *p53* embryos are developmentally normal? The *mdm2*/*p53* rescue phenomenon suggests that either p53 is not completely transcriptionally latent or that it has other activities which must be inactivated by mdm2 at the appropriate time in development. Although the first possibility cannot be ruled out, we would like to speculate that p53 actually has two activities in the developing embryo, one inducible and one constitutive (fig. 1). The inducible activity is the classic DNA damage or stress-induced transcriptional activation response. Activated p53 then transcriptionally regulates growth control genes (e.g., *p21*) or apoptosis regulatory genes (e.g., *bax*). The end result is growth arrest and apoptosis, which may both contribute to embryonic lethality.

The nature of the proposed constitutive activity is less clear, but may relate to some aspect of cell-cycle control or DNA replication. p53 has been shown to regulate DNA replication independent of its transcriptional regulatory capabilities [62]. Thus, at

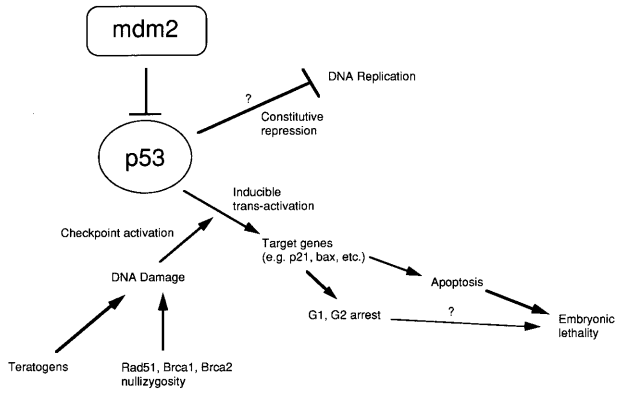

Figure 1. This model indicates key molecules and pathways in p53 regulation during early development. p53 is envisioned to have two activities during early development: (i) an inducible DNA damage response pathway; and (ii) a constitutive growth suppressive pathway which may directly modulate DNA replication. The question mark indicates the speculative nature of this hypothesized latter pathway. p53 levels are modulated in part by mdm2 binding and degradation. This activity is considered particularly important at about day 5.5 of gestation, when cell-cycle progression is rapidly accelerated. Teratogens and genetic defects in various DNA repair genes (*Rad51*, *Brca1*, *Brca2*) can activate the inducible p53 checkpoint function, which results in p53 stabilization and transcriptional activation of key target genes involved in growth arrest and apoptosis. Either cell-cycle arrest or apoptotic pathways are likely to induce embryonic lethality if sufficient numbers of cells are affected.

the critical developmental transition (at gestation day 5.5–6.5 in mice), when cell-cycle progression rates are dramatically accelerated, inactivation of this DNA replication-inhibiting activity by mdm2 could be essential. Recently, it has been shown that complexing of mdm2 with p53 facilitates degradation of p53, and this might be sufficient to allow the embryonic cells to increase their cell-cycle rates [63]. Suggestive evidence for this proposed constitutive embryonic p53 activity has been provided by Bernstein and colleagues, who showed that germ line transmission of an amino-truncated 44-kDa version of p53 (which would delete most of the transcriptional activation domain) could not be achieved despite extensive efforts [30]. This particular mutated *p53* should have been inactive as a transcription factor, yet it remained incompatible with embryonic development, consistent with the possibility of a second growth suppressive activity in the truncated molecule. Confirmation of this model, however, will clearly depend on generation of further mutant *p53* mice, particularly those that selectively inactivate the transcriptional regulatory domains.

In summary, a number of lines of evidence have led to the conclusion that p53 plays a highly important part in the development of the vertebrate organism in addition to its well-publicized role as a tumour suppressor. The next several years should result in further elucidation of the mechanisms by which p53 influences differentiation and development. Some of these insights are likely to have a critical impact on our perceptions of p53 as a tumour suppressor and may lead to novel cancer therapeutic and preventative approaches.

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