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Development of mice without Cip/Kip CDK inhibitors

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ABSTRACT

Timely exit of cells from the cell cycle is essential for proper cell differentiation during embryogenesis. Cyclin-dependent kinase (CDK) inhibitors (CKIs) of the Cip/Kip family (p21, p27, and p57) are negative regulators of cell cycle progression and are thought to be essential for development. However, the extent of functional redundancy among Cip/Kip family members has remained largely unknown. We have now generated mice that lack all three Cip/Kip CKIs (TKO mice) and compared them with those lacking each possible pair of these proteins (DKO mice). We found that the TKO embryos develop normally until midgestation but die around embryonic day (E) 13.5, slightly earlier than p27/p57 DKO embryos. The TKO embryos manifested morphological abnormalities as well as increased rates of cell proliferation and apoptosis in the placenta and lens that were essentially indistinguishable from those of p27/p57 DKO mice. Unexpectedly, the proliferation rate and cell cycle profile of mouse embryonic fibroblasts (MEFs) lacking all three Cip/Kip CKIs did not differ substantially from those of control MEFs. The abundance and kinase activity of CDK2 were markedly increased, whereas CDK4 activity and cyclin D1 abundance were decreased, in both p27/p57 DKO and TKO MEFs during progression from G_0 to S phase compared with those in control MEFs. The extents of the increase in CDK2 activity and the decrease in CDK4 activity and cyclin D1 abundance were greater in TKO MEFs than in p27/p57 DKO MEFs. These results suggest that p27 and p57 play an essential role in mouse development after midgestation, and that p21 plays only an auxiliary role in normal development (although it is thought to be a key player in the response to DNA damage).

1. Introduction

Cell proliferation is thought to be controlled by the balance between cyclin-dependent kinases (CDKs) and their inhibitors (CKIs). In general, CDKs promote cell cycle progression, whereas CKIs function to restrain it. CKIs bind to various cyclin–CDK complexes and thereby inhibit their kinase activities. They are categorized in two families on the basis of their structure and specificity for different cyclin–CDK complexes [1]. Members of the Ink4 family—$p16^{Ink4a}$ (p16), $p15^{Ink4b}$ (p15), $p18^{Ink4c}$ (p18), and $p19^{Ink4d}$ (p19)—are inhibitors specific for CDK4 or CDK6, whereas those of the Cip/Kip family, including $p21^{Cip1}$ (p21), $p27^{Kip1}$ (p27), and $p57^{Kip2}$ (p57), mainly target CDK2 and CDK4 or CDK6 (and CDK1 in some situations) for inhibition. In addition, all Cip/Kip CKIs promote the association of CDK4 with D-type cyclins [2].

Members of the Cip/Kip family share a conserved NH_2-terminal domain that contributes to association with and inhibition of cyclin–CDK complexes [1,3]. However, the remaining portions of the three proteins do not share sequence similarity, suggesting that Cip/Kip CKIs may have additional distinct functions or regulatory mechanisms. For example, transcription of the p21 gene is activated in response to expression of p53 induced by DNA damage, resulting in cell cycle arrest in G_1 or G_2 phases of the cell cycle. In contrast, expression of p27 is increased in mitogen-deprived cells or otherwise quiescent cells, and the protein is rapidly exported from the nucleus and degraded as cells enter the cell cycle. Unlike p21 and p27, p57 plays a specific role in embryonic development as well as in maintenance of stem cells in adults [4]. The

Abbreviations: CDK, cyclin-dependent kinase; CKI, CDK inhibitor; E, embryonic day; MEF, mouse embryonic fibroblast; RT, reverse transcription; PCR, polymerase chain reaction; ARBP, acidic ribosomal phosphoprotein; BrdU, bromodeoxyuridine; TUNEL, terminal deoxynucleotidyl transferase–mediated dUTP-biotin nick end labeling.

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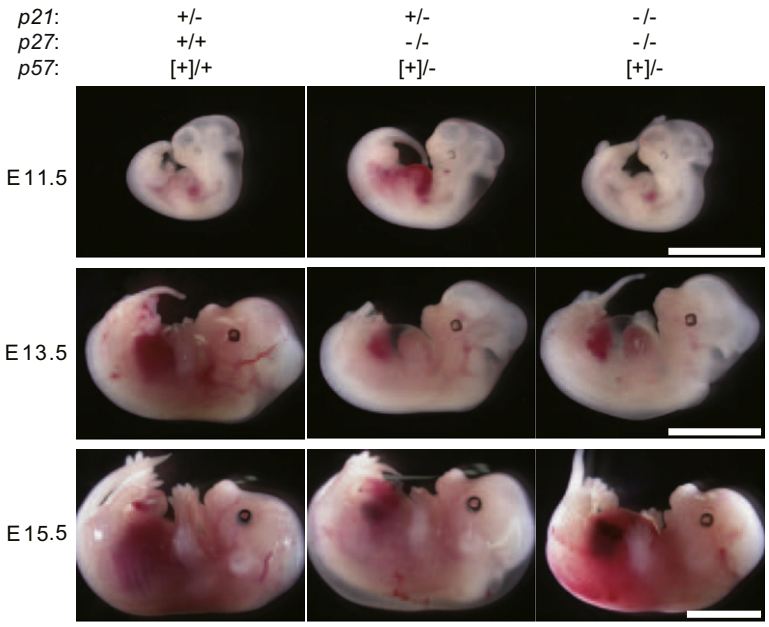
A

Stage	Observed (total)/expected Survival rate					Total
	$p21^{+/-}$ $p27^{+/+}$ $p57^{[+]/+}$	$p21^{-/-}$ $p27^{-/-}$ $p57^{[+]/+}$	$p21^{-/-}$ $p27^{+/+}$ $p57^{[+]/-}$	$p21^{+/-}$ $p27^{-/-}$ $p57^{[+]/-}$	$p21^{-/-}$ $p27^{-/-}$ $p57^{[+]/-}$	
E11.5	28 (29)/18.5 97%	19 (20)/18.5 95%	17 (17)/18.5 100%	8 (9)/18.5 89%	16 (20)/18.5 80%	296
E13.5	15 (15)/17.5 100%	19 (20)/17.5 95%	15 (17)/17.5 88%	11 (20)/17.5 55%	15 (21)/17.5 71%	280
E15.5	15 (15)/17.3 100%	14 (17)/17.3 82%	17 (21)/17.3 81%	3 (9)/17.3 33%	0 (20)/17.3 0%	277

B



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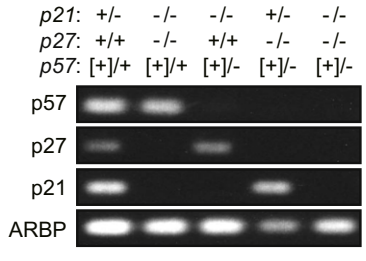

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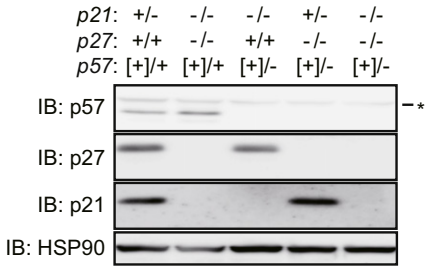

Fig. 1. Mice deficient in all Cip/Kip family CKIs die earlier than those deficient in both p27 and p57. (A) Summary of genotypes determined for embryos generated from intercrosses of $p21^{-/-}p27^{+/-}p57^{[+]/+}$ males with $p21^{+/-}p27^{+/-}p57^{[-]/+}$ females, or of $p21^{+/-}p27^{+/-}p57^{[+]/+}$ males with $p21^{-/-}p27^{+/-}p57^{[-]/+}$ females. Observed/expected numbers for each genotype are shown at E11.5, E13.5, and E15.5, with "observed" referring to the number of live embryos at the time of inspection; the total number of live and dead embryos of each genotype is indicated in parentheses. Survival rate was calculated as live/total embryos at each stage. (B) Representative appearance of E11.5, E13.5, and E15.5 embryos of the indicted genotypes. Scale bars, 5 mm. (C) RT-PCR analysis of p21, p27, p57, and ARBP (control) mRNAs in MEFs derived from embryos of the indicated genotypes. (D) Immunoblot (IB) analysis of p21, p27, p57, and heat shock protein 90 (HSP90, control) in the indicated MEF lines after incubation with the proteasome inhibitor MG132 (10 μM) for 8 h. Asterisk indicates nonspecific bands.