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RAPID COMMUNICATION

Induction of Cell-Cycle Inhibitor p21 in Rat Ventricular Myocytes during Early Postnatal Transition from Hyperplasia to Hypertrophy

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Summary

To examine a possible involvement of p21 protein, an inhibitor of cyclin-dependent kinases (CDKs), in the transition from hyperplastic to hypertrophic growth of rat ventricular myocytes during the first postnatal week, we analysed day-by-day changes in the number of p21 positive cells using specific antibodies against this protein. Paraffin-embedded sections of the left ventricular myocardium were examined by means of immunoperoxidase technique and hematoxylin-eosin counterstaining. While during the first three postnatal days, the positive reaction for p21 was detected only in a small fraction of myocytes (12–20 %), a sudden increase in positivity occurred on day 4 (54 %) and continued till day 6 when the fraction of cells expressing p21 reached 87 %. Our results show that the induction of CDK inhibitor p21 in rat ventricular myocytes is developmentally regulated. Moreover, the fact that the sudden increase in p21 positivity occurred at the same stage when the myocyte proliferation rapidly ceases, suggests that this protein is likely to be involved in mediating this key event of cardiac development.

Key words

Ventricular myocyte – Cell cycle – Proliferation – Postnatal development

Hyperplasia of mammalian ventricular cardiomyocytes is limited to the embryonic, foetal and early postnatal period of development. The exact stage at which myocytes cease to proliferate and become terminally differentiated is not clearly defined. The growth of rat ventricular myocytes has been divided into three distinct phases: i) hyperplastic phase from birth till postnatal day 6, ii) transitional phase between days 6 and 14, when hyperplasia and hypertrophy occur simultaneously, and iii) hypertrophic phase after day 14 (Clubb and Bishop 1984). Others, however, suggested that myocyte proliferation takes place even beyond this stage (e.g. Claycomb 1975). In contrast, a recent study indicated, based on bromodeoxyuridine incorporation and morphometric analysis, that the transitional period is very short and a rapid switch of myocytes from hyperplastic to hypertrophic growth occurs already between days 3 and 4 of postnatal life (Li *et al.* 1996). Unlike the other cell types, ventricular myocytes retain limited capacity to replicate DNA without subsequent cell division, resulting in formation of binucleated and multinucleated cells. In the rat, DNA synthesis ceases between postnatal days 10 and 17 which corresponds to the completion of cell binucleation (Claycomb 1975, Li *et al.* 1996).

The reason why ventricular myocytes lose their ability to divide during normal maturation and the molecular mechanisms controlling this process remain

largely unknown (for review see McGill and Brooks 1995). Negative control of mammalian cell-cycle progression involves a family of proteins known as inhibitors of cyclin-dependent kinases (CDKs). The assembly and activation of complexes of these CDKs and cyclins is required for progression to the next step of cell cycle (for review see Hartwell and Kastan 1994, Lees 1995). The induction of CDK inhibitors appears to be a contributing mechanism by which cells irreversibly exit the cell cycle upon terminal differentiation. The first member of this family to be identified and cloned was p21 which is the product of the WAF1/CIP1/SDI1 gene (El-Deiry *et al.* 1993, Harper *et al.* 1993). The p21 binds to and inhibits the activity of a broad range of cyclin-CDK complexes (Xiong *et al.* 1993) and thus it may control both the G_2/M and the G_1 cell cycle checkpoints (Agarwal *et al.* 1995). In addition, this protein inhibits DNA synthesis through interaction with proliferating cell-nuclear antigen (PCNA), the essential DNA replication factor which activates DNA polymerase δ (Waga *et al.* 1994). These two antiproliferative mechanisms of p21 action seem to be independently executed by separate protein domains (Luo *et al.* 1995).

To study a possible involvement of p21 in the transition from hyperplastic to hypertrophic growth, we analysed day-by-day changes in p21 labelling of rat ventricular myocytes during the first postnatal week. The left ventricular myocardium of Wistar rats was sampled on postnatal days 1–7. Paraffin-embedded sections were examined for p21 by means of an immunoperoxidase technique. Monoclonal antibody WAF1 which recognizes C-terminal region of p21, has been developed in our laboratory. In order to distinguish the cardiac myocytes from non-myocyte cells, the sections were counterstained with hematoxylin-eosin.

As shown in Figure 1, in the myocardium of 1, 2 and 3-day-old animals, the positive nuclear reaction for p21 was observed only in a small fraction of myocytes (12–20 %). An abrupt increase in positivity to 54 % occurred on postnatal day 4 and continued till day 6 when 87 % of myocytes exhibited positive reaction.

Our data demonstrate that the induction of CDK inhibitor p21 in cardiac myocytes is developmentally regulated. The sudden increase in p21 positivity between postnatal days 3 and 4 is in agreement with the data of Li *et al.* (1996) indicating that a rapid termination of hyperplastic myocyte growth occurs exactly at the same stage. Thus, it appears likely that p21 may be involved in mediating this key event of postnatally developing heart. It has been shown that PCNA, one of the target compounds for p21 antiproliferative effect, is still expressed in rat ventricular myocytes at this stage (Marino *et al.* 1991) and about 15 % of cells are PCNA positive even on day 12 (Heron *et al.* 1997). Moreover, cyclin A, the complexes of which with CDKs are inhibited by p21, also disappears only during the second postnatal week (Yoshizumi *et al.* 1995).

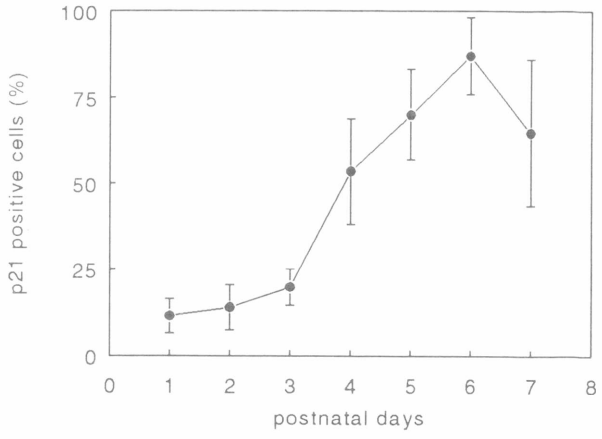

Fig. 1. *The percentage of left ventricular myocytes expressing p21 protein in rats during the first postnatal week. Each point represents the mean ± S.D. of 4 hearts. In each heart, 200 randomly selected cells were evaluated.*

The possible mechanism of p21 induction in the developing ventricular myocytes is unclear. The expression of gene encoding this protein is generally induced by the tumor-suppressor protein p53 which binds to the p21 promoter (El-Deiry *et al.* 1995). During cell differentiation and senescence, however, the expression of p21 appears to be stimulated independently of p53 function (MacLeod *et al.* 1995). Terminal cell cycle arrest during murine skeletal muscle differentiation correlated with p21 induction, mediated by a specific transcriptional regulator MyoD that did not require p53 (Halevy *et al.* 1995). In some cells, the p21 can also be induced through p53-independent mechanisms by growth factors and protein kinase C activating substances, like phorbol esters (Michieli *et al.* 1994, Tchou *et al.* 1996). In accordance with these observations, we did not detect any change in p53 expression in the rat ventricular tissue during the first postnatal week (data not shown). Understanding the precise regulatory mechanism of p21 and its role in the neonatal myocytes requires further investigation.

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Reprint requests

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