Expression of Cellular Oncogenes in the Myocardium During the Developmental Stage and Pressure-Overloaded Hypertrophy of the Rat Heart

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Proto-oncogenes have been revealed to participate in normal cell proliferation as well as in cell transformation. Since cardiac myocytes are terminally differentiated, they cannot divide except in the fetal period. To determine the role of cellular oncogenes in the growth of the heart, the expression pattern of eight cellular oncogenes during the developmental stage and pressure-overloaded hypertrophy of the rat hearts were examined in vivo. Northern blot analysis was performed with eight oncogene probes (myc, fos, Ha-ras, src, erbA, erbB, sis, myb). Pressure overload increased the levels of cellular (c-) fos, c-myc, and c-Ha-ras. An increase of c-fos and c-myc was detected at 30 minutes and 2 hours, respectively; the levels peaked at 8 hours, and they returned to baseline by 48 hours after aortic constriction. However, the level of c-Ha-ras showed a gradual increase. During the course of development, the expression of c-myc was detectable only in the embryonic stage, whereas the expression of c-fos was not detected in the fetal period, was increased after birth, and peaked in 200-day-old adults. The expression of c-Ha-ras was almost the same throughout the development. Cellular oncogenes were expressed in the heart in response to pressure overload and in a stage-specific manner. These results suggest that cellular oncogenes may participate in the normal developmental process and hypertrophy of hearts and that the cellular hypertrophy induced by pressure overload may share a similar mechanistic pathway with cell proliferation. (Circulation Research 1988;62:1075–1079)

Various types of malignancies can be induced by the RNA tumor viruses, and these retroviruses are known to carry specific genes, which are designated viral oncogenes.1 Cellular oncogenes (c-onc), homologous to retroviral oncogenes, have been hypothesized to play a role in growth control. Recent evidence that the products of several proto-oncogenes are either growth factors2 or growth factor receptors3 strongly suggested this hypothesis. Much information has accumulated that pathological expression of c-onc is related to cell transformation in vitro and malignant disease in vivo; however, very little is known about the role of c-onc in the physiology of normal cells.

Cardiac myocytes can divide only in the fetal period, and soon after birth, they lose their ability to replicate DNA. After birth, hearts grow by an increase in cell size (hypertrophy), not in cell number (proliferation). Recently, the induction of cardiac myocyte hypertrophy has been reported to be associated with enhanced expression of the c-myc gene by exposure to α1-adrenergic agonists in the culture cells.4 In the present study, we examined the expression of c-onc genes in the rat heart during the developmental stage and during hypertrophy caused by pressure overload.

Materials and Methods

Animals and Surgical Procedures

To produce pressure-overloaded cardiac hypertrophy, male Wistar rats, which were 40 days old and weighed 180–200 g, were anesthetized with diethyl ether, and the upper part of the abdominal aorta was constricted with a hemoclip. Ninety-one of the 110 operated rats survived the procedures and were killed at predetermined times after the operation (0.5, 2, 4, 8, 12, 24, 48, and 72 hours). Sham-operated animals underwent an identical procedure except for placement of the hemoclip and were killed at different time intervals postoperatively. The mean aortic pressure in the aortic-constricted animals increased 46 ± 5 mm Hg compared with the sham-operated controls (n = 3). To investigate the developmental change, hearts of 12-, 15-, and 18-day-old embryos, 5-day-old neonates, and 40- and 200-day-old adults were examined.

RNA Preparation

Hearts were excised, and the atria, great vessels, and right ventricular free walls were removed. The left ventricles were rinsed with cold saline and quickly frozen in liquid nitrogen. Total cellular RNA was extracted from the myocardium by the lithium urea method.3 Poly-A+ RNA was enriched by oligo(dT)-cellulose chromatography.
Hybridization Analyses

Poly-A⁺ RNA (3 μg) was denatured at 60°C for 7 minutes, fractionated by electrophoresis through 1.2% agarose gels, and transferred to nylon membranes. The membranes were exposed to ultraviolet rays for 2.5 minutes, prehybridized, and hybridized at 42°C with 32P-labeled oncogene probes: human c-myc, exon III, EcoR I/Cla I; v-fos, Bgl II/Pvu II; v-Ha-ras, Bal I/Sal I; v-erbA, Pst I/Pst I; v-erbB, BamHI/EcoR I; v-sis, Pst I/Pst I; v-myb, Sal I/Sal I; and v-src, Pvu II/Pvu II. The human c-myc probe was a generous gift of Dr. T. Sekiya, National Cancer Center, Tokyo. Other viral oncogenes were obtained from the Japanese Cancer Research Resources Bank, Tokyo. Prehybridization was performed in a solution containing 5 X SSPE (saline-sodium-phosphate-EDTA) buffer, 5 X Denhardt's solution, 1% sodium dodecyl sulfate (SDS), 10% dextran sulphate, and 100 μg/ml heat-denatured salmon sperm DNA for 6-12 hours at 42°C. Hybridization was performed in the same solution with the addition of 5 x 10⁶ cpm/ml 32P-labeled probe for 24-36 hours at 42°C. Probes were prepared with the random-priming procedures. Membranes were washed twice at 42°C with 2 X SSC (saline sodium citrate) buffer and 0.1% SDS, twice at 42°C with 0.2 X SSC and 0.1% SDS, air dried, and exposed to x-ray film (Kodak XAR-5) for 24 hours or 5 days with an intensifying screen at -70°C. Relative amounts of c-onc expression were determined by a densitometric scanner.

Results

RNA Blot Hybridization Analysis of c-onc in Pressure-Overloaded Cardiac Hypertrophy

Figure 1 shows Northern blot analysis of three oncogenes, c-fos, c-myc, and c-Ha-ras, which were expressed at appreciable levels in rat hearts because of pressure overload. Since in the normal adult hearts, these oncogenes were either slightly or not expressed, the sample that was pressure-overloaded for 8 hours is used in the figure. The expression of the other five c-onc were not detected in this study. Figure 2 shows the expression patterns of these three genes during pressure overload. Sham operation did not change the expression levels of these genes. The increase of the expression of c-fos-related sequences was recognized as early as 30 minutes after operation. Two peaks were

![Figure 1](http://circres.ahajournals.org/) RNA blot hybridization analysis of three c-onc genes in rat hearts. Poly-A⁺ RNA (3 μg) of the rat heart, which was pressure-overloaded for 8 hours, was separated on a 1.2% agarose gel, blotted onto a nylon membrane, and hybridized to a random-priming probe (v-fos, c-myc, and c-Ha-ras). The autoradiograms were exposed for 24 hours with intensifying screen at -70°C. Size of rRNA is indicated as marker.
observed, one at 30 minutes and one at 8 hours after operation. By densitometric scanning, the more prominent peak at 8 hours after operation showed an 8- to 10-fold increase over the level of the preoperative control (Figure 3). The level of the expression was gradually decreased thereafter to the baseline at 48 hours. The expression pattern of c-myc was similar to that of c-fos. Its expression was detectable by 2 hours, peaked at 8 hours, and decreased to baseline at 48 hours after operation. Comparison of relative levels of the expression by densitometric scanning of the autoradiograms showed an 8- to 10-fold increase in the c-myc-related message at 8 hours compared with 2 hours after operation. Some c-Ha-ras-related sequences were expressed in sham-operated hearts and increased gradually by pressure overload. Densitometric scanning revealed a threefold to fivefold increase of c-Ha-ras-related sequences at 48 hours, as compared with the preoperative controls (Figure 3).

RNA Blot Hybridization Analysis of c-onc During Development of Rat Heart

Three of the same genes were also detected. Sequences that were c-myc related were detectable in the fetal period but disappeared soon after birth. Relative levels of the expression determined by densitometric scanning of the autoradiograms were almost the same throughout the embryonic stage. In contrast, embryonic hearts showed no detectable c-fos transcripts. An increase of the expression of the sequences related to the c-fos gene was detected at the neonatal period and a gradual increase continued and peaked in 200-day-old adults. The expression of c-Ha-ras-related sequences was detected throughout the developmental stage, and its level was not changed among three stages.

Discussion

Evidence has accumulated that normal cellular proliferation is controlled by the interaction of several...
classes of proto-oncogenes and that changes in the expression of controlling elements of normal proliferation result in the uncontrolled cellular growth. In the present study, we have examined the relation between cardiac growth and proto-oncogene expression in rat hearts. Among the eight oncogenes investigated, three oncogenes were expressed at measurable levels. An increase of c-fos and c-myc was detected at 30 minutes and 2 hours, respectively, and the levels peaked at 8 hours and returned to baseline by 48 hours after aortic constriction. The expression of c-Ha-ras, however, was detected from the preoperative hearts and increased gradually by pressure overload. During the course of heart development, the expression of c-fos was detected after birth and increased gradually, whereas that of c-myc was detected only in the fetal stage. The level of c-Ha-ras was not changed throughout the development.

The c-fos is the cellular homologue of the oncogene of two mouse osteosarcoma viruses, FBJ-MSV and FBR-MSV. All fos genes encode nuclear proteins and show a complex pattern of tissue-, cell type-, and stage-specific expression, suggesting a correlation with cellular differentiation and proliferation. The increase of c-fos was detected as early as 30 minutes, peaked at 8 hours, and returned to the uninduced level by 48 hours after aortic constriction. In the pressure-overloaded cardiac myocardium, not only is total protein content increased, but also different types of proteins are synthesized. For instance, the isozymic transition of myosin heavy chains was induced by 24 hours after aortic constriction (data not shown), and in the other contractile proteins, actin or tropomyosin, expression of fetal isoforms was reported in the hypertrophic hearts. Therefore, the proteins, such as c-fos, that were increased in the early stage of pressure overload appear to play an important role in cardiac hypertrophy. During development, the level of c-fos expression was very low in the fetal periods and only gradually increased. Although the role of c-fos in the heart is unknown, its pattern of expression is distinctive and of interest. Further study is necessary to clarify the role of c-fos with regard to proliferation and differentiation processes during cardiac hypertrophy and aging.

The oncogene c-myc also encodes nuclear protein, is expressed in relation to the cell cycle, and may play a role in cellular proliferation. Recently, the expression of c-myc was reported to be increased in cultured cardiac myocytes and in induced hypertrophy by α-adrenergic agents. In the present study, the c-myc gene was also expressed in pressure-overloaded hearts in vivo. The c-myc gene was expressed in the fetal heart, which is in the proliferative period. Cardiac myocytes are terminally differentiated and cannot divide after birth. The c-myc gene, which was expressed only in the fetal heart in the physiological state, was reintroduced in adult heart by pressure overload, suggesting that the cell hypertrophy induced by pressure overload may share a common mechanistic pathway with cell proliferation and that enhanced expression of the c-myc gene may be related to both cardiac cell division and cell hypertrophy.

The Ha-ras genes encode 21-kDa proteins that appear to be involved in the control of cellular growth and differentiation, and mutations affecting ras and...
overproduction of normal 21-kDa proteins can induce a transformed phenotype. Recently, 21-kDa proteins were reported to affect both the phosphatidylinositol-4,5-bisphosphate breakdown pathway and the level of inositol-1,4,5-trisphosphate that would be elevated in ras-transformed cells. In cultured cardiac myocytes, α1-adrenergic agonists have been reported to induce hypertrophy through the phosphoinositide/protein kinase C pathway. In this study, mRNA encoding of the ras gene was highly expressed in the pressure-overloaded heart. These results and observations suggest that enhanced expression of the ras gene might be associated with cardiac hypertrophy.

In cardiac hypertrophy, c-fos and c-myc genes were expressed from as early as 30 minutes and 2 hours after pressure overload, respectively. Morkin and Ashford reported that there was little change in DNA synthesis in the interstitial cells of pressure-overloaded hypertrophic hearts until the 2nd postoperative day. Furthermore, the c-myc gene was recently demonstrated to be expressed in cultured cardiac myocytes in the induction of hypertrophy. Although oncogenes were reported to be expressed in fibroblasts or other nonmuscle cells in the proliferative period, together with these observations, it is possible to speculate that these cellular oncogenes were expressed in cardiac myocytes. But further investigation is needed to know the cellular origin of these changes and the relation between cellular oncogenes and cardiac hypertrophy.

Acknowledgments

We are indebted to Drs. Takao Sekiya and Yoshinori Murakami, National Cancer Center, Tokyo, for providing human c-myc probes. We would like to thank Miss Kazue Minamisako for her excellent technical assistance.

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Key Words • cardiac hypertrophy • cardiac development • cellular oncogenes
Expression of cellular oncogenes in the myocardium during the developmental stage and pressure-overloaded hypertrophy of the rat heart.
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doi: 10.1161/01.RES.62.6.1075

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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http://circres.ahajournals.org/content/62/6/1075

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