Acta Med. Nagasaki 34 : 173−176

Immunohistochemical Demonstration of myc Gene Product in Chick Embryo Aorta and Atherosclerotic Lesions in Chickens

Takayoshi TODA

Depertment of Clinical Laboratory, Univerysity Hospital, School of Medicine, University of the Ryukyus, Okinawa 903, Japan

Received for publication, December 26, 1987

SUMMARY : Expression of c-myc oncogene was immunohistochemically examined on aortas of chick embryo of 2-week gestational age, 2-week-old normal cockerels, 4-month-old normal roosters and 4-month-old cholesterl-fed roosters. Chick embryo showed moderate levels of expression of the c-myc oncogene product in the entire wall of the aortas. There was no significant expression of c-myc oncogenic protein in the thickened intima and the tunica media of the ascending aortic arch and its large branches of the cockerels and normal roosters. Cholesterol-fed roosters showed marked expression of c-myc oncogenic protein in the lipid-rich thickened intimal lesions of the ascending aortic arch and its large branches. These results suggest that c-myc oncogene has a role in proliferation and differentiation of aortic medial cells as well as development of atherosclerosis in chickens.

INTRODUCTION

Atherosclerosis is a multifactorial disease which affects medium-sized arteries and large arteries. Recent studies have indicated that proliferation of intimal smooth muscle cells is one of the key factors in the development of atherosclerosis (2,8). Ross *et al.* (8) suggested that chronic cycles and repair are responsible for the development of atherosclerosis, and that injury is exacerbated by risk factors for atherosclerosis such as hypertension, hypercholesterolemia, and cigarettes smoking. On the other hand, the monoclonal hypothesis was proposed by Benditt *et al.* (2) that atherosclerotic plaque represents a benign neoplasm originated from a virus or chemical-induced cells. Penn *et al.* (5) have found that transforming gene, which is different from v-Ki-ras, N-ras and v-Ha-ras genes, exists in human atherosclerotic plaque DNA. Previously, we have reported that several oncogene products are expressed in human coronary atherosclerotic lesions (10) as well as in thyroid malignant tumor (11). These results seem to support the idea that interactions between many different cellular events, such as localized systems of growth factors and transactivation of tissue-specific gene regulation operating during embryogenesis (6). The purpose of the present paper is to document myc oncogene expression in the developing chick aorta and atherosclerotic lesions of the cholesterol-fed chickens.

MATERIALS AND METHODS

Three chick embryos, 2 weeks of gestational age, were fixed in 10 per cent formalin solution. Serial sections of 4 micrometer-thicknes were made to locate the ascending aortic arch and its large branches of the chick embryo. The ascending aorta and its large branches and the heart were also obtained from five 2-week-old normal cockerels, and three 4-month-old

normal roosters. Five 1-month-old cockerels were fed with an atherogenic diet containing 2 per cent of cholesterol and 10 per cent of corn oil for 3 months. Tissues were fixed in 10 per cent formalin solution and embedded in paraffin. The paraffin sections were cut into 4-mirometer thickness and stained with hematoxylin-eosin, Mallory azan, and elastica van Gieson. Immunoperoxidase staining was performed on sectioned tissues by the peroxidase -antiperoxidase (PAP) method of Sternberger *et al.* (9). Sections were treated with 0.3 per cent hydrogen peroxide to block endogenous peroxidase. After exposure to non-immune serum, the sections were reacted with either the primary anti-sera or non-immune sera, link antibodies, followed by peroxidase-anti-peroxidase complex for 1 hour at room temperature. The sections were washed three times with 0.1M phosphate buffered solution (PH 7.5) for 5 minutes after each antibody application and were treated with diamino-bendizine in hydrogen peroxide. The primary antibody for myc oncogenic protein (Oncr. Inc. Wako) was used in this study. Serum cholesterol was determined by the enzymatic method (TC-S 736, Kyowa, Tokyo).

RESULTS

At the age of 2 weeks' gestation, the ascending aortas consisted of spindle cells. Myc oncogene product was seen scattered in numerous nuclei of spindle cells in the tunica media (Fig. 1). Serum levels of cholesterol ranged from 137 to 193mg/dl in normal 2-week-old cockarels and 4-month -old roosters. In the aortas of 2-week-old cockerels, two types of cells including smooth muscle cells and fibroblasts were identified in the tunica media. Intimal thickening was not significantly seen (Fig. 2). Some medial smooth muscle cells showed weak reaction to the anti-bodies for myc oncogene product. The ascending aortic arch and its large branches of 4-month-old rooster had medial architecture with alternating layer of muscular and non-muscular lamellae and slight intimal thickening (Fig. 3). However, no significant amount of myc oncoprotein was demonstrated in all layers of the ascending aortic arch and its large branches.

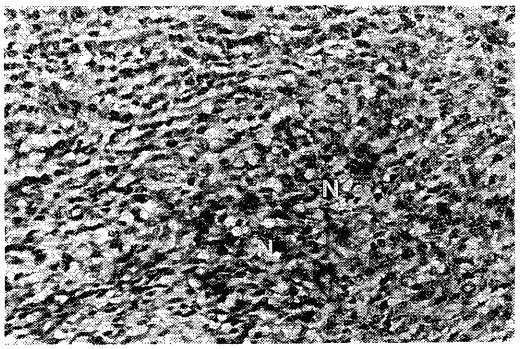

Fig. 1. Note nuclear oncoprotein staining in spindle cells (N) scattered in the entire wall of the aorta from a chick embryo of 2 weeks' gestational age (PAP stain×180).

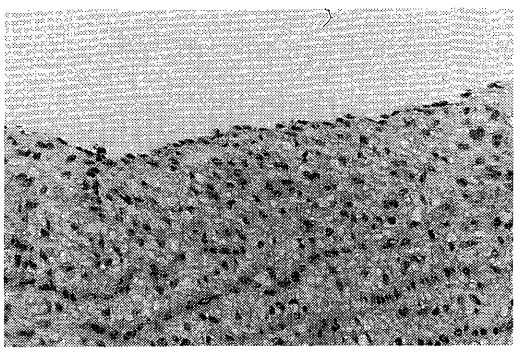

Fig. 2. Ascending aortic arch without intimal thickening from a 2 weeks old cockerel (Hematoxylin and eosin stain ×180).

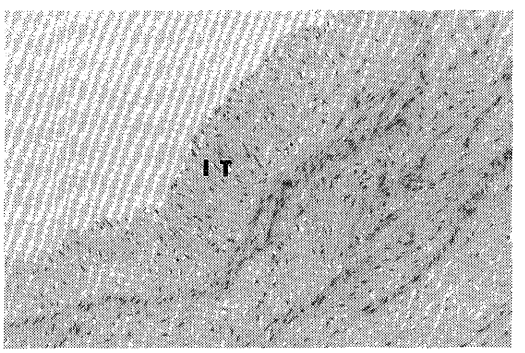

Fig. 3. Ascending aortic arch with slight intimal thickening (IT) from a 4 months old rooster (Hematoxylin and eosin stain ×180).

Serum levels of cholesterol ranged from 655

to 1806 mg/dl in 4-month-old roosters fed with cholesterol. Three months of cholesterol feeding produced marked cellular intimal thickeking with lipid deposition in the ascending aortic arch and its large branches (Fig. 4). Myc oncoprotein was intensely demonstrated especially in foam cells of lipid-rich intimal lesions, but not in the tunica media of the ascending aortic arch and its large branches (Fig. 5).

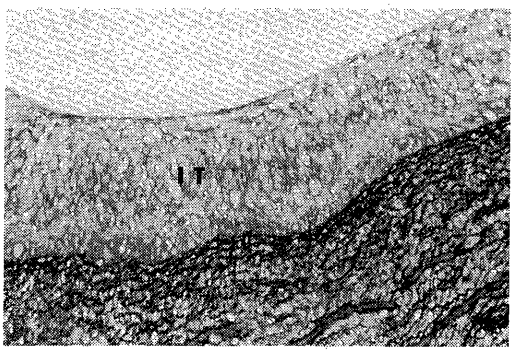

Fig. 4. Ascending aortic arch with marked intimal thickening (IT) from a cholesterol-fed rooster (Elastica van Gieson stain × 180).

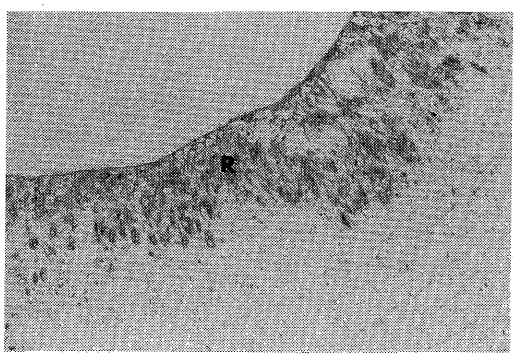

Fig. 5. Lipid-rich aortic lesions from a cholesterol-fed rooster showing intense reaction (R) for antibody of myc oncoprotein (PAP stain ×180).

DISCUSSION

Lachman *et al.* (4) reported that myc oncogene was first identified in retroviruses and has a regulatory function in the control of cell growth. Studies of c-myc gene expression in a variety of cell types and tissues have suggested that the c-myc gene was rearranged, amplified, and overexpressed in a wide variety of human cancers (1, 3, 7).

Zimmerman *et al.* (12) examined the expression of N-myc, L-myc and C-myc in murine newborn and adult tissues including brain, kidney, adrenal, thymus, spleen, liver, intestine, lung and heart. They also suggested that differential expression of several genes may be important in normal develpoment and predicted the types of tumors in which they are expressed or activated.

The present result demonstrates that myc oncoprotein is present in the aorta of chick embryo and the lipid-rich intimal lesions of the ascending aortic arch and its branches, but not in the tunica media of the aorta. It is clear that the myc oncogene has an important role in normal embryogenesis of the arterial system and atherosclerotic process. Further quantitaive, DNA, and RNA studies are required to elucidate how proto-oncogenes or oncogenes are involved in atherogenesis.

A part of this work was presented at the 77 th annual meeting of Japan Pathological Society in Hokkaido in 1988.

REFERENCES

1) Alitalo, K. and Schwab, M. : Oncogene amplification in tumor cells. Adv. *Cancer Res.* **47** : 235-281 (1986).
2) Benditt, E. P. and Benditt, J. M. : Evidence for a monoclonal origin of human atherosclerotic plaques. Proc Natl Acad Sci. *USA.* **70** : 1753-1756 (1973).
3) Erisman, M. D., Rothberg, P. G., Diehl, R. E., Morse C. C., Spandorfer, J. M., and Astrin, S. M. : Deregulation of c-myc gene expression in human colon carcinoma is not accompanied by amplification or rearrangement of the gene. *Mol. Cel. Biol.* **5** : 1969-1976 (1985).
4) Lachman, H. M. and Skoultchi, A. I. : Expressionof c-myc changes during differentiation of mouse erythroleukemia cells. *Nature.* **310** : 592-594 (1984).
5) Penn, A., Garte, S. J., Warren, L., Nesta, D., and Mindich, B. : Transforming gene in human atherosclerotic plaque DNA. *Proc Natl Acad Sci. USA.* **83** : 7951-7955 (1986).

6) Pfeiffer-OHSSON, S., RYDNERT, J., GOSTIN, A. S., LARSON, E., BETSHOLTZ, C., and OHLSSON, R. : Celll-type-specific pattern of myc proto-oncogene expression in developing human embryos. *Proc. Natl. Acad. Sci. USA.* **82** : 5050-5054 (1985).

7) RIOU, G., LE, M. G. LEDOUSSAL, V., BARROIS, M., GEORGE, M., and HAIE, C. : C-myc proto-oncogene expression and prognosis in early carcinoma of the uterine cervix. *Lancet.* **1** : 761-763 (1987).

8) ROSS, R. : Atherosclerosis : A problem of the biology of arteral wall cells and their interaction with blood components. *Arteriosclerosis.* **1** : 293-311 (1988).

9) STERNBERGER, L. A., HARDY, P. H., CUCULIS, J. J., and MEYER, H. G. : The unlabeled antibody enzyme method of immunohistochemistry preparation and properties of soluble antigen and antibody complex (horseradish-antihorseradish peroxidase) and its use in identification of spirochetes. J. *Histochem Cytochem.* **18** : 315-333 (1970).

10) TODA, T., NAGAMINE, M., SAEKI, N., TAKEI, H., and HOKAMA, S. : Atherosclerosis as a proliferative disease of arterial intimal cells : An ultrastructural, immunohistochemical, and in situ hybridization study. *Acta Med. Nagasaki.* (in press).

11) TODA, T., DEGUCHI, S., KURIHARA, K., HAMADA, Y., MUTO, Y., and KITSUKAWA, K. : Expression of EGF, ras and myc oncogene products in normal, Graves' thyroid, adenoma, and carcinoma of the thyroid. *Acta Med Nagasaki.* (in press).

12) ZIMMERMAN, K. A., YANCOPOULOS, G. D., COLLUM, R. G., SMITH, R. K., KOHL, N. E., DENNIS, K. A., NAU, M. M., WITTE, O. N., TORAN-ALLERAND, D., GEE, C. E., MINNA, J. D., and ALT, F. W. : Differential expression of myc family genes during murine development. *Nature.* **319** : 789-783 (1986).