PROTO-ONCOGENE EXPRESSION IN THE MALE RAT LIVER AT DIFFERENT AGES

L. B. Novikov, R. M. Balanskii, V. N. Anisimov, and K. P. Khanson*

UDC 616.006.6.092:577.73-083.72

KEY WORDS: proto-oncogenes; liver; aging; rat

More and more evidence has recently been obtained that the genetic lesions which determine aging and the development of neoplasms are essentially similar [5, 9]. Cellular proto-oncogenes may play a key role in their realization. First, they are involved in normal biological processes such as the regulation of division, growth, and differentiation, and second, their activation leads to malignant transformation and promotes tumor progression [4]. Since the frequency of neoplasms is known to increase with age, and sensitivity of the body to the action of carcinogenic factors of different nature also changes significantly with age, it is interesting to study proto-oncogene expression in animal tissues during aging.

The aim of this investigation was to study expression of ten proto-oncogenes in the liver of male rats aged between 1 and 37 months.

EXPERIMENTAL METHOD

Proto-oncogene expression was evaluated quantitatively by detecting molecules of specific mRNA contained in the total cell RNA pool obtained from the liver of male LIO rats aged 1, 10.5, 22, and 37 months [2]. RNA preparations isolated from the liver by the method described previously [3] were applied to nitrocellulose filters in 4 dilutions by a "Minifold" apparatus (Schleicher und Schuell," West Germany). The filters were hybridized with the following tumor-specific DNA probes: v-Ha-ras (plasmid pBS-9), v-myc (pv-myc), v-yes (pv-yes), v-fos (pv-fos), v-mos (pMS-1), v-ki-ras (pHi-Hi 3), v-sis (pv-sis), v-abI (pAB 3 sub 3), human c-met (pmet D) and human N-myc (pNB-1). Prehybridization and hybridization of the filters was carried out at 60-62°C for 4 and 24 h respectively, in buffer containing 3 × SSC, 0.1% SDS, 0.5 mg/ml yeast tRNA, 5 × Denhardt's reagent, and DNA probe with specific radioactivity of $2 \cdot 10^8$ cpm/μg, labeled in the nick-translation reaction with ^{32}P-αdCTP pBq/mole ("Amersham," England, and "Izotop," USSR). The filters were washed to remove unhybridized DNA probe in a solution of 2 × SSC, 0.1% SDS at room temperature, 2 × SSC, 0.1% SDS at 37°C, and 0.1 × SSC, 0.1% SDS at 50°C. For autoradiography the filters were exposed on x-ray film (ORWO, East Germany) in casettes with intensifying screens for 20 days. The level of proto-oncogene expression was determined on a "Diamed" FLR-1D/2D laser densitometer (USA), establishing the degree of irradiation of the x-ray film resulting from dot hybridization of radioactively labeled DNA probes with preparations of cellular RNA applied to the filters.

EXPERIMENTAL RESULTS

The dot hybridization method was used for quantitative evaluation of proto-oncogene expression in the rat liver because its sensitivity attains one transcript per cell and enables a difference of twice or more in gene expression to be

*Corresponding Member of the Academy of Medical Sciences of the USSR.

Laboratory of Biochemistry and Laboratory of Experimental Tumors, Professor N. N. Petrov Research Institute of Oncology, Ministry of Health of the USSR, Leningrad. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 5, pp. 521-522, May, 1991. Original article submitted June 29, 1990.

0007-4888/91/0005-0679$12.50

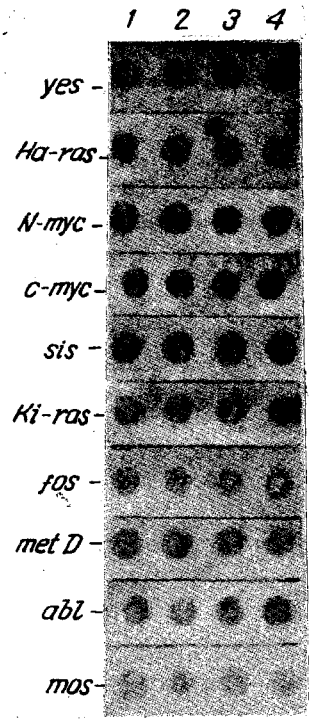

Fig. 1. Analysis of proto-oncogene expression by dot hybridization of ^{32}P-dCTP-labeled DNA probes with preparations of total cellular RNA of the liver of LIO rats aged 1 month (1), 10.5 months (2), 22 months (3), and 37 months (4).

detected [12]. To reach such high sensitivity of the method, we did not use formamide in the hybridization reaction. This was because neither of the DNA probes in our possession (viral and human oncogenes) is strictly complimentary to the corresponding rat genes. We know that the denaturing action of formamide consists of its ability to bind with free H_2-groups of adenine, preventing the formation of A–T pairs during hybridization [2]. Addition of formamide to the hybridization mixture would lead to an even greater decrease in the percentage of homology between radioactively labeled DNA probes and transcripts of the test rat proto-oncogenes, which would reduce the number of DNA–RNA duplexes in the hybridization dot.

The study of proto-oncogene expression showed that the efficiency of hybridization of each DNA probe used with preparations of total liver cellular RNA did not change with age of the animal (Fig. 1). This indicates that the number of transcripts and, consequently, the level of expression of proto-oncogenes C-MYC, C-FOS, N-MYC, HA-RAS, KI-RAS, SIS, ABL, YES, MOS, and MET in the liver tissue of LIO rats remains the same throughout the first 37 months of their life (from 1 to 37 months of age) and does not change during growth of the organ. A similar result was obtained by a study of proto-oncogene expression in the liver of Buffalo rats [13]. The authors cited showed by dot hybridization that the number of HA-RAS, ERB B, MYS, and FOS transcripts in the liver falls sharply during the first month of life, after which it remains at the same level, and during development of the animals from fetus to adult expression of SRC, FMS, REL, MOS, SIS, MYB, and KI-RAS proto-oncogenes in the liver is virtually unchanged. In C57BL/6NJcl mice reduction of expression of the C-MYC proto-oncogene in the liver takes place during the first 14 months of life and remains unchanged until the age of 26 months [8]. A different pattern of proto-oncogene expression is found in the liver of F-344 rats: a gradually increasing number of C-MYC transcripts until the age of 14 months followed by maintenance of constant transcription activity of SIS and SRC for 22 months [7]. Expression of the C-MYC proto-oncogene falls sharply during the first 2 months of life, but starting from the age of 6 months the number of C-MYC transcripts increases whereas the number of N-MYC

and L-MYC transcripts remains unchanged throughout the animals' life [9]. There is reason to suppose that every line of animals (rats and mice) has its own age-related pattern of proto-oncogene expression.

A study of the oncologic characteristics of LIO rats showed [4] that spontaneous tumors of the pituitary gland, hematopoietic system, and mammary gland (in females) appear most frequently in these animals, and tumors of the thyroid, adrenals, gonads, skin, soft tissues, bone, lungs, and small intestine less frequently. Development of spontaneous liver tumors has not been observed in LIO rats, whereas in intact old F-344 rats the development of preneoplastic and neoplastic changes is observed in the liver and they are highly sensitive to the action of promoters of hepatocarcinogenesis [9, 11]. As a result, analysis of expression of SIS, N-RAS, MOS, SRC, C-MYC, HA-RAS, C-FOS, KI-RAS, and ABL in different organs of intact LIO rats aged 1-1.5 years showed [1] that the number of transcripts of the above-mentioned proto-oncogenes in their liver was appreciably less than in cells of the brain, heart, gastric mucosa, striated muscle tissue, lungs, and white blood cells. The possibility cannot be ruled out that definite correlation exists between the transcription activity of proto-oncogenes and tissue sensitivity to spontaneous and induced carcinogenesis.

Thus the number of transcripts of C-MYC, C-FOS, N-MYC, HA-RAS, KI-RAS, SIS, ABL, YES, MOS, and MET proto-oncogenes in the liver of male LIO rats does not change during the period from 1 to 37 months of age, and it is this feature which may perhaps determine the resistance of these animals to spontaneous hepatocarcinogenesis.

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