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SELECTIVE ELEVATION OF C-MYC TRANSCRIPT LEVELS IN THE LIVER OF THE AGING FISCHER-344 RAT

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SUMMARY: The expression of several proto-oncogenes involved in normal cell growth was examined as a function of age in male Fischer-344 rats aged 3, 6-9, 12 and 21-23 months. Northern blot analysis using RNA isolated from pooled livers or brains showed that the transcript levels of c-myc, but not c-sis or c-src-related genes, were markedly elevated in the liver with age. In contrast, there was no substantial change in transcript levels of any of these genes in aging brain. When c-myc expression was analyzed in livers from individual rats ranging in age from 4 to 22 months, a significant ($p<0.01$) 5-fold increase in c-myc transcript levels in relation to age was detected. The results indicate that expression of c-myc in rat liver is modulated during aging and more generally, that aging in rats is associated with organ-specific changes in the transcript levels of particular genes.

During cell differentiation, gene transcription becomes increasingly restricted. Although this is a major tenet of development, it is not obvious whether the expression of specific genes in stabilized throughout the lifespan. Several theories on the biology of aging suggest there is a continuum of molecular changes between cells in differentiating and senescent states (1) and therefore, predict that gene expression is modulated not only during development, but during cellular aging as well.

Genes that may undergo age-related changes are those involved in regulation of cell growth, such as the proto-oncogenes (c-onc genes). These genes encode proteins associated with DNA binding, protein-tyrosine phosphorylation, and several proteins related to growth factors and growth factor receptors (2). Because multiple regulatory roles have been implicated for the c-onc genes, we postulated that expression of at least some of these genes may be altered during normal cellular aging. Here we test this hypothesis by measuring the transcript levels of several c-onc genes in brain and liver of the Fischer-344 rat in relation to age.

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MATERIALS AND METHODS

Animals. Two groups of male Fischer-344 rats were utilized, aged 3, 6-9, 12 and 21-23 months, and aged 4, 14 and 22 months. All animals were obtained from Charles River Laboratories (Kingston, MA).

RNA Isolation and Hybridization Analysis. Total RNA was isolated from whole livers or brains using a guanidine thiocyanate/lithium chloride procedure (3). The polyadenylated fractions were selected by oligodeoxythymidine cellulose chromatography. Equal amounts of the polyadenylated RNAs were denatured in 6.5% formaldehyde and 50% formamide and size-fractionated by electrophoresis in 1% agarose gels containing 6.5% formaldehyde (4). The gels were stained with ethidium bromide to confirm the integrity of the different RNA preparations and then subjected to Northern blotting onto nylon membranes (Bio-Rad). Hybridization was carried out as described by Church and Gilbert (5) using [^{32}P]-labeled DNA probes. Hybridization signals were visualized by autoradiography using enhancing screens and band intensity was measured by densitometry (2202 Ultroscan Laser Densitometer, LKB). Transcript sizes in kilobase pairs were estimated relative to the 18S and 28S ribosomal RNAs remaining after one pass over the oligodeoxythymidine cellulose column, and with E. coli ribosomal RNA standards. Significant differences in hybridization intensity was determined using the Kruskal-Wallis test (6).

DNA Probes. The following probes were nick-translated to 10^7-10^9 cpm/μg of DNA using [^{32}P]dCTP: [A] the 0.98-kb Sst I-Xba I fragment of v-sis (7); [B] the 0.8-kb Pvu II fragment of v-src (8); and [C] the 1.8-kb Cla I-Eco RI 3rd exon of human c-myc (9).

RESULTS

The expression of c-onc transcript levels in the aging rat brain and liver was examined by Northern blot hybridization. Initially, we screened for several-fold differences between groups of aging animals, and for the specificity of changes by examining c-onc genes of different functional subclasses. These were c-sis, which encodes the B-subunit of platelet derived growth factor (PDGF) (10); c-src, whose product is a protein-tyrosine kinase involved in cell metabolic processes (11); and c-myc, which encodes a nuclear protein that may have a role in DNA synthesis (12).

Northern blot analysis of c-sis was carried out using a fragment of v-sis as a probe; the sequence homology between the cellular and viral forms of this gene has been described (7). As shown in Fig. 1, a predominant 4.1-kb band was present in brain (lanes 3-6), and its size agreed with that of c-sis-related transcripts seen in other tissues (13). The c-sis gene is expressed primarily in cells of mesenchymal origin. In brain, c-sis transcripts are likely associated with the glial population, since these cells are mesenchyme-derived, possess PDGF receptors, and show a mitogenic response to PDGF (14). Further, c-sis transcripts have been detected in glioblastomas (13). Densitometric analysis showed that the intensity of 4.1-kb band did not vary by more than 1.6-fold among the various age groups, indicating that expression of c-sis in brain did not change markedly with age. A

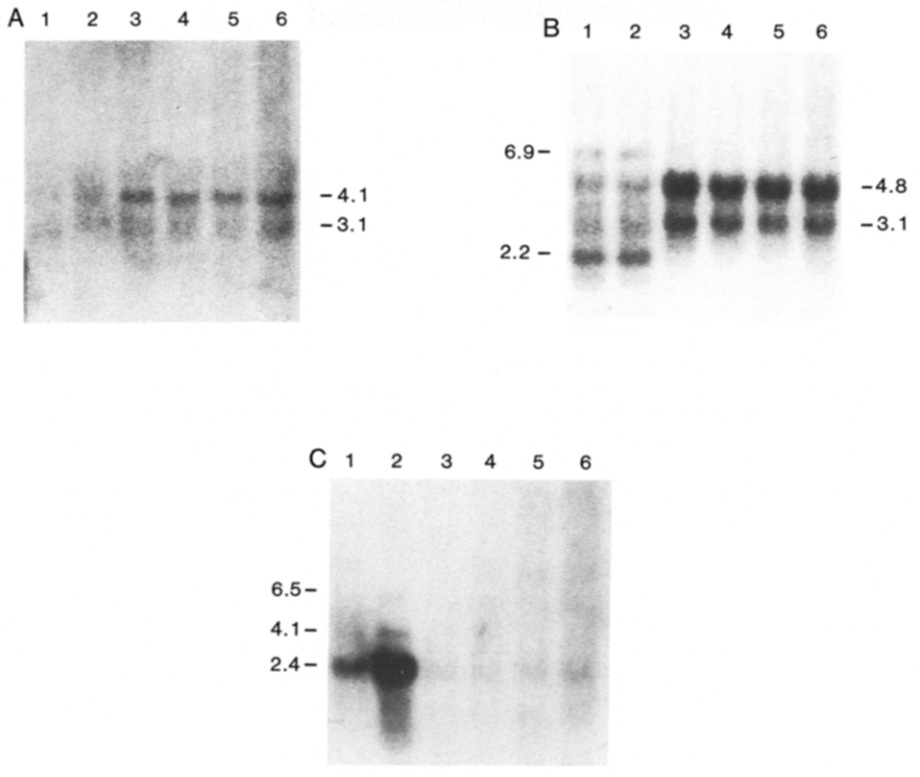

Fig. 1. Expression of c-onc transcripts in brain and liver of Fischer-344 rats aged 3, 6-9, 12 and 21-23 months. Equal amounts (15 µg) of polyadenylated RNAs were electrophoresed in denaturing gels, blotted to nylon membranes and hybridized with one of the following [^{32}P]-labeled DNA probes: [A] v-sis; [B] v-src; [C] c-myc. Each lane represents RNA isolated from organs pooled from several animals in each age group. Transcript sizes are given in kilobase pairs. Lanes 1 and 2, RNA from liver of rats aged 6-9 and 21-23 months, respectively; lanes 3-6, RNA from brain of rats aged 3, 6-9, 12 and 21-23 months, respectively.

weakly-hybridizing band of 3.1-kb also was apparent. The nature of this band is not known, but minor c-sis-related transcripts have been reported (13). Hybridization of the probe to the RNAs from liver was barely detectable (lanes 1 and 2); a longer exposure of the blot indicated the presence of transcripts similar in size to those seen in brain and that there was no substantial difference in their intensity with age.

Analysis of c-src transcripts was carried out using a v-src probe that encodes the tyrosine kinase domain of the gene (15). This region of the src protein has sequence homology to other protein-tyrosine kinases, which include both receptors for growth factors and non-receptor type oncogenes. Homology is most pronounced between the products of the c-src and c-yes (16), c-slc/syn (17), c-fgr (18), lyn (19), and lsk/tck (20,21) genes, indicating they make up a closely-related family of protein-tyrosine kinases. Accordingly, the v-src probe hybridized to several c-src-related transcripts (Fig. 1B). The overall band intensity was greater in brain (lanes 3-6) than in liver (lanes 1 and 2),

consistent with the relative over-expression of protein-tyrosine kinases in neural tissues (19, 22). The predominant bands detected in brain corresponded to mRNA species of 4.8- and 3.1-kb, whose sizes are similar to the human c-src (23) and the c-slc/syn (17) transcripts, respectively. In addition to the major bands detected in brain, 6.9- and 2.2-kb bands were present exclusively in liver. Presently, we cannot identify these transcripts, because a 6.9-kb mRNA has not been reported for any of the described protein-tyrosine kinases and, although the 2.2-kb band corresponds in size to the lsk/tck mRNA, previous studies in the mouse indicate that this gene is not expressed in liver (21). The levels of the transcripts in liver did not differ by more than 1.3-fold between the 6-9 and 21-23 month animals. In brain, the levels of the 4.8- and 3.1-kb transcripts were 1.2-fold and 1.6-fold higher, respectively, in the 3 month animals as compared to the 6-9 month animals and thereafter remained approximately the same among the 6-9, 12 and 21-23 month animals. These observations suggest that the expression of the individual c-src-related genes detected with this probe is relatively stable in the liver and brain during aging of the rat.

Hybridization analysis using the c-myc probe (Fig. 1C) detected the 2.4-kb transcript of the c-myc gene (24). The band intensity was elevated markedly in the pooled livers from the 21-23 month animals (lane 2) relative to the 6-9 month animals (lane 1), and the over-expression of c-myc in the older rats precluded an accurate measurement of this change. Also, there was an age-related appearance of 6.5- and 4.1-kb bands whose sizes were similar to those of the nuclear precursors of c-myc cytoplasmic RNA. In contrast to liver, brain had less than a 2-fold increase in the levels of the c-myc transcript in relation to age (lanes 3-6). The same filter was hybridized with a beta actin probe in order to confirm that the age-associated change in c-myc expression was not due to degradation or differential blotting of the RNA. In this case, there was no substantial change in the transcript levels of the beta actin gene with age in either liver or brain (data not shown).

These data indicate that the mRNA levels of c-sis, c-myc and c-src-related genes are relatively stable in brain, whereas in liver, there is a selective elevation of c-myc mRNA in relation to age. We wished to accurately measure this change, and therefore, Northern blot analysis was carried out using liver polyadenylated RNAs isolated from 9 individual rats aged 4, 14 or 22 months ($n = 3$ per age group). As shown in Fig. 2, the level of the 2.4-kb transcript in the 22 month animals was approximately 3.5- to 8-fold higher than the mean level detected in the 4 month animals. At least one higher molecular weight transcript (4.0 kb) was detected in the older animals. Overall, there was a significant 5-fold and 2.4-fold difference, respectively, in c-myc expression between the 22 and 4 month animals ($p<0.01$) and the 22 and 14 month animals ($p<0.05$).

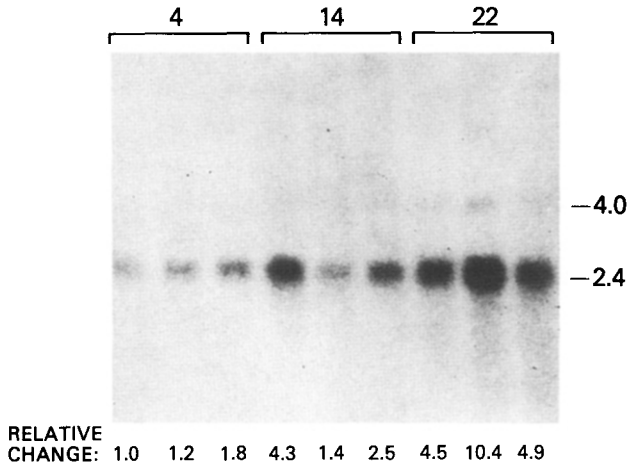

Fig. 2. Expression of c-myc transcripts in liver of rats aged 4, 14 or 22 months. The polyadenylated RNAs were electrophoresed (5 μg per lane) and subjected to Northern blotting and hybridization using a [^{32}P]-labeled c-myc probe. The animal age corresponding to the RNA sample is given at the top of the figure. The bottom of the figure indicates the change in hybridization intensity of each lane relative to the first lane. Each lane represents RNA isolated from a single animal. Transcript sizes are given in kilobase pairs.

DISCUSSION

Although it is not known how c-myc mRNA levels are elevated with age in the rat liver, cell transformation is probably not involved. There was no gross evidence of tumors in these animals, and moreover, hepatic neoplasms are uncommon in the senescent Fischer-344 rat (25). Also, the change does not appear to reflect the age-associated polypoidy commonly seen in hepatocyte nuclei (26), as there was not a corresponding increase in c-sis or c-src-related transcripts. Still, the possible role of polypoidy cannot be excluded until cells showing increased levels of c-myc mRNA are identified using combined methods of in situ hybridization and histochemistry.

Observations in cell culture systems have established that transcript levels of mature c-myc increase in response to mitogen-induced cell proliferation, and that they decrease or are undetectable in cells terminally differentiated or arrested in G_0-G_1 (27). Further, the increase can be inhibited by anti-mitogenic agents such as interferons (28). These data underscore the importance of growth factors in the regulation of this gene, and it is likely that they influence c-myc expression during normal aging. In the regenerating rat liver, c-myc transcript levels are also elevated, albeit transiently, within hours after partial hepatectomy (29). This change is specific, because transcript levels of c-src, c-abl, or c-mos do not concomitantly increase. Although aging and regeneration of liver are very different processes, the preferential increase in c-myc expression in both

might suggest that the hormonal factors essential for organ regeneration (30) may also have a role in aging. Hormones needed for regenerating liver include glucagon, insulin and epidermal growth factor (EGF); of these, EGF has been shown to induce c-myc transcript levels in vitro (31). On the other hand, it is possible that the altered c-myc expression in the aging liver is due to an environmental influence or an age-related loss of a negative regulator. Determining what factors modulate c-myc during normal cellular aging may give further insight into the regulation of this gene.

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