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EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR PROTO-ONCOGENE mRNA IN REGENERATING RAT LIVER

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SUMMARY: The expression of EGF receptor mRNAs in regenerating rat liver was measured using two nonoverlapping cDNA probes for the human gene from a highly conserved region. These probes (pE7 and pE62) both hybridized to RNA species of 10 and 6 kb. The 10 and 6 kb RNA species were shown to decrease in the first 12 hours after partial hepatectomy. However, significant increases above control levels were noted at 24h and 72h. The level of α-actin mRNA increased as has been previously reported. These results suggest that a transcriptional and/or a posttranscriptional regulatory mechanism exists in regenerating rat liver with respect to EGF receptor gene expression.

INTRODUCTION: The mechanism by which epidermal growth factor (EGF) stimulates cell proliferation is not well understood. The cell surface receptor that is required for the action of EGF contains an amino portion that binds EGF, a transmembrane domain and a kinase domain (1,2,3); the latter two domains are homologous to the viral erbB oncogene protein product (4,5). The number of EGF receptors is often increased in malignant cell lines derived from human cancers (6). The level of EGF receptor mRNA is also frequently elevated in tumors and cell lines derived from tumors (7,8,9,10,11,12). Furthermore, increasing the number of EGF receptors in NIH/3T3 cells using a retrovirus carrying the EGF receptor gene leads to a transformed phenotype dependent on the presence of EGF (13). It has also been reported that EGF down regulates the number of EGF receptors on the cell surface (14) and increases the expression of EGF receptor mRNAs (15,16,17).

Since EGF plays an important role in cell proliferation, it is likely that the regulation of the EGF receptor in a highly proliferative system such

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as regenerating liver is very important. Although it has not been directly shown that EGF plays a major role in liver regeneration in vivo, several lines of suggestive evidence do exist. EGF stimulates DNA synthesis in normal liver (18). Hepatocytes take up a very high percentage (>90%) of labeled EGF in one pass through the liver (19). Measurement of EGF receptor levels by EGF binding and immunochemical techniques shows a decrease in receptor in the early hours after partial hepatectomy (20,21). Later the level of EGF has been found to rise in the plasma of hepatectomized rats (22).

Since recent studies have shown that EGF regulates the level of EGF receptor mRNA in cultured cells (15,16,17) and the concentration of EGF in plasma rises after partial hepatectomy, we analyzed the expression of EGF receptor mRNA in regenerating rat liver using cDNA probes from the human gene (23).

METHODS

Animal and Treatment Protocol. Male Fisher rats, weighing 150 to 175 grams, were obtained from the National Institute of Health Animal Supply (Bethesda, MD) and were maintained on purina rat chow and water ad libitum with alternating 12 hour periods of light and darkness. Partial hepatectomy were performed under halothane anethesia by the method of Higgins and Anderson (24).

RNA Isolation. Tissue were removed and immediately frozen in liquid nitrogen and then ground fine with a mortar and pestle. RNA was isolated by the guanidine thiocynate method used by Schweizer and Goerttler (25), and enriched for poly(A) RNA by two successive cycles of Oligo(dT)-cellulose chromatography (26).

RNA Blot Analysis. Electrophoresis of poly(A) RNA samples on horizontal agarose gels were performed as previously described (27). Membranes were hybridized to [^{32}P]-nick translated DNA probes (28) by the method of Thomas (29). After hybridization, blots were washed at room temperature (23°C) with 2SCC/0.1% SDS for 30 minutes with a total of two changes, 2SSC/0.1% SDS at 50°C for 30 min with a total of two changes and finally with 0.2SSC/0.1% SDS at room temperature for 30 min with a total of two changes. Filters were air dried and exposed to Kodak XAR-5 film with intensifying screens.

RESULTS AND DISCUSSION: To determine if changes in the level of EGF receptor mRNA occurred during liver regeneration, we isolated mRNAs at different times after partial hepatectomy and analyzed them by Northern blot hybridization using two cDNA probes for the human gene. The cDNA probe designated pE7 contains a 2.4 kb insert that codes for the transmembrane domain and the N-terminal portion of the kinase domain (23). Hybridization of regenerating rat liver RNA blots with this probe resulted in detection of three RNA

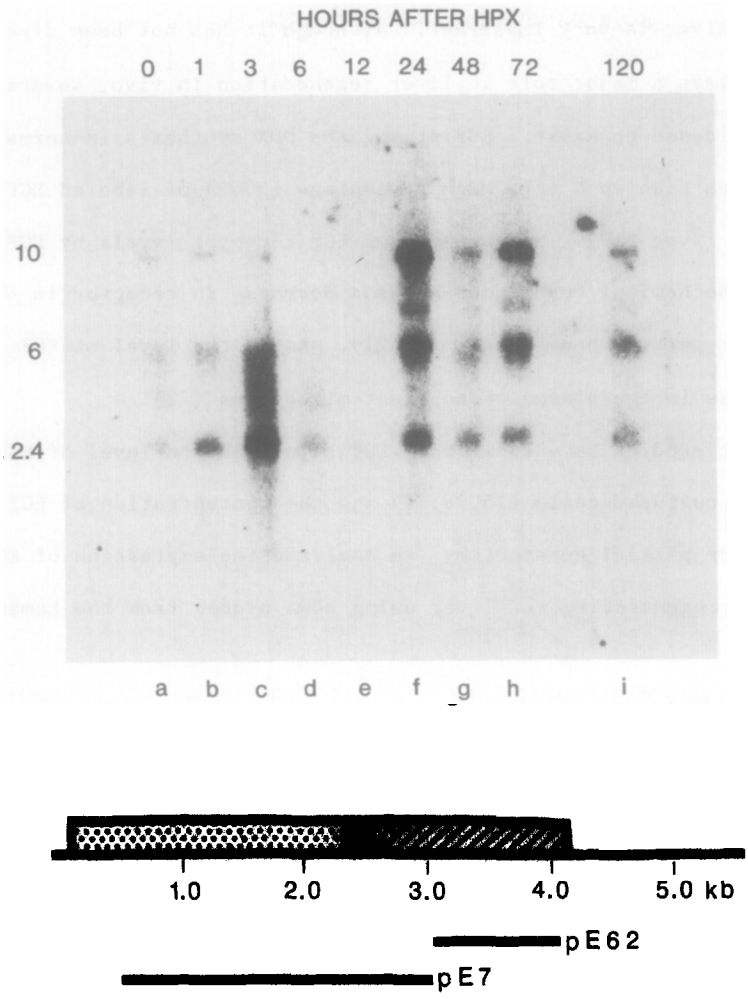

Figure 1. Northern blot hybridization analysis of regenerating rat liver poly(A) RNA with EGF receptor cDNA probe pE7. RNAs isolated from rat liver at times shown above the blots we hybridized with a nick-translated pE7 insert probe and washed as described in Methods. Sizes were determined using an RNA ladder from Bethesda Research Labs, Bethesda, MD. Sizes of mRNAs are shown to the left of the figure in kilobases. A schematic showing the location of the cDNA probes used in this study is illustrated beneath the figure. Amino portion containing the EGF binding domain. Transmembrane domain. Carboxyl portion that contains the kinase domain.

species; 10, 6 and 2.4 kb (Fig. 1). The expression of the 10 and 6 kb RNA species is low in normal rat liver (Fig. 1, lane a) and does not change in 1 and 3 h regenerating liver (Fig. 1, lanes b and c). EGF receptor mRNA levels are dramatically decreased in 6 h and 12 h regenerating liver (Table 1. Fig. 1, lanes d and e). A dramatic increase is detected in 24 h regenerating liver (Fig. 1, lane f). These RNA species are also elevated in 72 h regenerating liver (Fig. 1, lane h) and to a lesser extent at 120 h (Fig. 1, lane i). At

TABLE 1. CHANGES IN EGF RECEPTOR mRNA IN REGENERATING RAT LIVER

Hour After HPX	EGFR mRNA*
0	1.0
1	0.9 ± 0.1
3	0.9 ± 0.1
6	0.4 ± 0.2
12	0.2 ± 0.1
24	15.9 ± 4.6
48	1.6 ± 0.6
72	9.2 ± 4.0
120	3.3 ± 0.3

*Levels of RNA are relative to the 0 hr control and are expressed as mean ± std. deviation. Quantitation was performed by laser scanning densitometry of the 10 kb RNA species.

48 h after partial hepatectomy, these RNA species are about equal to the control level (Fig. 1, lane g). On the other hand, the 2.4 kb species does not follow the same pattern of expression and is possibly an RNA from another gene undergoing cross-hybridization.

To examine this possibility, we hybridized regenerating rat liver RNA blots with a nonoverlapping cDNA probe designated pE62. This cDNA contains a 1 kb insert specific for the c-terminal portion of the kinase domain of the EGF receptor and a portion of the 3' noncoding region (23). Hybridization of northern blots revealed the presence of the 10 and 6 kb RNA species but not the 2.4 kb species (Fig. 2). These RNA species were elevated at 24 h and 72 h (lanes b and c) but not at the earlier time points (1, 3, 6 and 12 h, data not shown). This result confirms that EGF receptor mRNA is increased in regenerating rat liver 24 and 72 h following partial hepatectomy. It is interesting that the sizes of the rat EGF receptor mRNA (10 and 6 kb) are very similar to those found in human cells (10 and 5.6 kb). RNA species of 10 and 6 kb have previously been identified in mouse cells (30). The 2.4 RNA species that hybridizes to the pE7 probe may encode a protein that has an amino portion or transmembrane domain similar to that of the EGF receptor.

As a control for these experiments, the regenerating rat liver RNA blots were hybridized with a probe for Y-actin mRNA. Actin mRNA was found to increase during the early hours after partial hepatectomy, but then decrease to only 2 to 4-fold over control levels at later time points (Fig. 3). The result reported here for actin is very similar to an earlier report (31). The

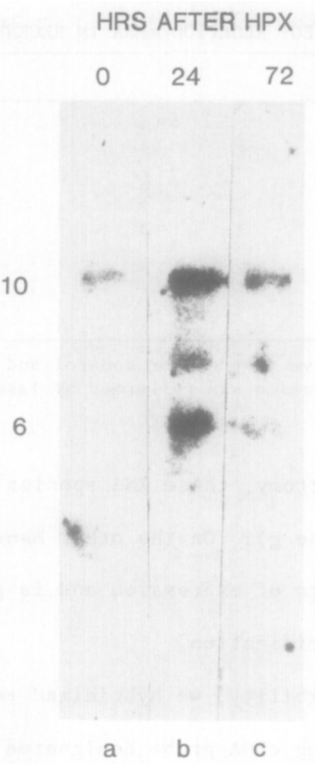

Figure 2. Northern blot hybridization analysis with an EGF receptor cDNA probe (pE62) specific for the homologous kinase domain. Analysis was as described in figure 1. The nick-translated insert of pE62 was hybridized and washed as described in Methods. Sizes of mRNAs are shown to the left of the figure in kilobases.

kinetics of actin mRNA expression and EGF receptor mRNA expression do not coincide and therefore are probably due to different signals. This result confirms that the induction of EGF receptor mRNAs is not due to either different amounts of RNA loaded onto the gel or differential transfer of specific lanes.

The finding that EGF receptor mRNAs are elevated in regenerating rat liver is not unexpected, since EGF concentrations are increased in the plasma of rats following partial hepatectomy (22), and EGF has been shown to increase EGF receptor mRNA in human cells (15,16,17). The exact mechanism by which EGF receptor mRNA is increased remains to be elucidated.

Two other proto-oncogenes, c-myc and c-fos, have previously been found to be increased in regenerating rat liver (31,32). Also, a multidrug resistance gene (MDR-1) is increased in regenerating rat liver and is often increased after chemical hepatocarcinogenesis (33). Characterization of the mechanism

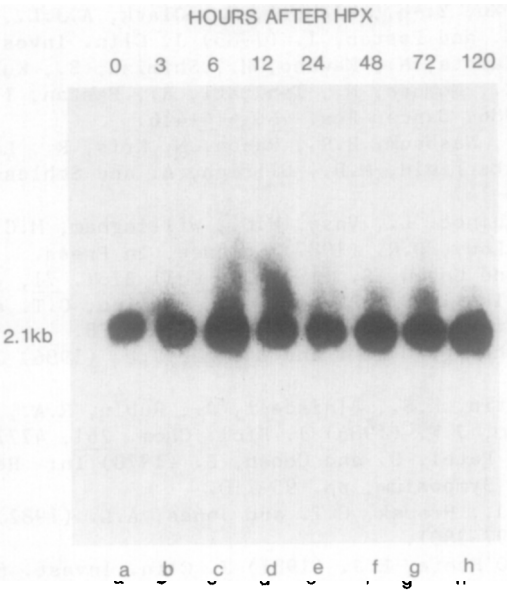

Figure 3. Northern blot hybridization analysis with an actin cDNA probe. A human actin probe (29), (pHFYA-1), was hybridized to regenerating rat liver blots and washed as described in Methods. Size of mRNA is shown to the left.

by which these genes are regulated in regenerating liver may help explain how increased expression of these genes occurs in cells transformed to malignancy.

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