LEVELS OF *c-K-ras* AND *hst-1/k-FGF* GENE PRODUCTS AND EGF IN REGENERATING RAT LIVER AFTER PARTIAL HEPATECTOMY

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ABSTRACT

As a part of our serial work on cell proliferation/growth-related gene products in regenerating rat liver after partial hepatectomy, we examined quantitative changes of *c-K-ras* product (*c-K-ras*-P), *hst-1/k-FGF* product (*hst-1/k-FGF*-P) and EGF during rat liver regeneration, using radioimmunoassays specific for the respective proteins. Immunologically measurable *c-K-ras*-P (*c-K-ras*-P-IR) was found to decrease significantly on days 3-7 of regeneration, while immunoreactive rat EGF (rEGF-IR) level was first raised markedly at 3, 6 and 12 h after resection and then lowered significantly on days 2-9 and 14 of regeneration. The present radioimmunoassay for *hst-1/k-FGF*-P revealed the presence of an immunoreactive component (*hst-1/k-FGF*-P-IR) of 17 kDa in normal and regenerating rat liver. However, *hst-1/k-FGF*-P-IR level in regenerating rat liver showed no significant changes during 14 days after partial hepatectomy. The concomitant decreases in *c-K-ras*-P-IR and rEGF-IR during the later stages of liver regeneration are assumed to relate to the repression mechanism of hepatocyte proliferation and growth after restoration of the residual liver. The early increase in rEGF-IR is supposed to reflect the amplified mRNA expression of EGF receptor during the same period of regeneration as has been described by others. The different patterns of the concentration change vs time curves of *c-K-ras*-P-IR, rEGF-IR and *hst-1/k-FGF*-P-IR in regenerating rat liver together with those of *c-myc*, *c-fos* and *RB* gene products immunologically measured in our previous studies suggest significant participation of these proteins by integrated coordination of the respective unique roles in cell proliferation and growth in regenerating rat liver after partial hepatectomy. Gomisin A exhibited lowering effects on the decreased levels of *c-K-ras*-P-IR, rEGF-IR as well as that of *RB* gene product radioimmunologically measured in the later stages of regeneration, while the level of *hst-1/k-FGF*-P-IR was not affected by the drug during 14 days after partial hepatectomy. These observations provided additional evidence for the growth-promoting activity of gomisin A on hepatocytes during regeneration of partially resected rat liver.

Several cell proliferation/growth-related genes, i.e. *c-myc*, *c-fos*, *c-ras*, *c-raf-1* and *A-raf-1*, have been shown to be activated in the immediate-early stage of rat liver regeneration after partial hepatectomy (10, 18, 21, 22, 25, 29, 37, 53, 55, 58). The products of *c-myc* (*c-myc*-P) and *c-fos* (*c-fos*-P) are both nuclear proteins having DNA binding activity. The

retinoblastoma susceptibility gene (*RB*) has been recognized as a growth suppressor gene and its product (*RB*-P) is another nuclear protein with DNA binding activity possessing a function implicated in normal cell growth (4, 6, 35). Our previous study has shown that *c-myc*-P and *c-fos*-P levels in regenerating rat liver measured by specific radioim-

munoassays (*c-myc*-P-IR and *c-fos*-P-IR, respectively) increased significantly during days 1–6 of regeneration after partial resection (30). We have also demonstrated that radioimmunologically measurable *RB*-P (*RB*-P-IR) in regenerating rat liver decreased, inversely to the increase in *c-myc*-P-IR, during days 1–4 of regeneration after partial hepatectomy (31). In this study, we extended our work aiming to explore the implication and significance of cell proliferation/growth-related gene products in rat liver regeneration after partial hepatectomy to quantitative analyses, using specific radioimmunoassays, of *c-K-ras* product (*c-K-ras*-P) and *hst-1/k-FGF* product (*hst-1/k-FGF*-P) in regenerating liver. Epidermal growth factor (EGF) was also examined by a radioimmunoassay specific for rat EGF, since EGF has been shown to act as a stimulus for hepatocyte DNA synthesis during liver regeneration (40), and to participate in the expression of *c-ras* and activation of the gene products in various cell lines (7–9, 27, 36, 41, 48, 49, 60). We have previously demonstrated that gomisin A, a dibenzocyclooctadiene type lignan, had an enlarging effect on mean hepatocyte cell size on days 3 and 4 and an amplifying effect on *c-myc*-P-IR increase on days 5–8 of regeneration of partially resected rat liver and suggested a growth-promoting activity of the drug on hepatocytes during regeneration (24). In the present study, the concentration changes in radioimmunologically measurable *c-K-ras*-P (*c-K-ras*-P-IR), *hst-1/k-FGF*-P (*hst-1/k-FGF*-P-IR) and rat EGF (rEGF-IR) as well as *RB*-P-IR in regenerating liver of gomisin A-treated rats were also examined.

MATERIALS AND METHODS

Drug

Gomisin A was a generous gift from Tsumura (Tokyo), which had been proved to have 99% purity.

Animals

Adult male Wistar rats (Nihon Dobutsu, Osaka) weighing 180–200 g were used. Rats were maintained for 1 week on diet MF (Oriental Yeast, Tokyo). Animals were anesthetized with ether and subjected to partial (70%) hepatectomy or sham-operation according to the method of Higgins and Anderson (23). Twenty-four hours after operation, gomisin A (100 mg/kg body weight) in 0.5% aqueous CMC-Na (1 ml/100 g body weight) was given orally once a day for 14 days. Rats of control group

were given vehicle (0.5% aqueous CMC-Na).

Tissue Extraction

The animals including sham- and non-operated controls were anesthetized with ether and the liver was removed. After weighing and washing with ice-cold saline, the organs were immediately frozen. The liver was homogenized with a Bio-Mixer model BM-1 (Nihon Seiki Seisakusho, Tokyo) in an ice-bath in a 5-fold weight of ice-cold 50 mM Tris-HCl buffer (pH 7.5) containing 5% glycerol, 50 mM NaCl, 1 mM phenylmethylsulfonylfluoride (PMSF) (Sigma Chemicals, St. Louis, MO, U.S.A.) and 250 KIU/ml aprotinin (Bayer, Leuven, Germany). The homogenate was centrifuged at 160,000 g for 60 min at 4°C in a Beckman L8-60M ultracentrifuge and the supernatant was stored at –30°C. Protein concentration was determined by the method of Bradford (5).

Gel Filtration

Gel filtration of rat liver extract was carried out on a column (1.6 × 96 cm) of Sephacryl S-200 superfine (Sephacryl 6B for *RB*-P) using, as eluent, 50 mM Tris-HCl buffer (pH 7.5) containing 50 mM NaCl, 50 mM EDTA, 1 mM PMSF and 0.02% NaN₃. Fractions of 2.0 ml each (3.7 ml for *RB*-P measurement) were collected and subjected to radioimmunoassays. For rat EGF analysis, gel filtration was performed on a column of Sephadex G50 superfine (1.6 × 64 cm) with 1 M acetic acid as eluent.

Immunoblot Analysis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were carried out as described previously (30) according to the methods of Laemmli (32) and Towbin and coworkers (59), respectively, with slight modifications. Antisera R0811 for *RB*-P, R4203 for *c-K-ras*-P, R0681 for *hst-1/k-FGF*-P and SAR301 for rat EGF were used in immunoblot analysis.

Radioimmunoassays

Radioimmunoassays for *RB*-P, *c-K-ras*-P, *hst-1/k-FGF*-P and rat EGF were carried out according to the method previously described for *c-myc*-P measurement (30).

***RB*-P** The reagents used were a synthetic pep-

Fig. 1 Typical standard curves (—●—) of radioimmunoassays for c-K-ras-P-IR (a), *hst-1/k-FGF*-P-IR (b) and rEGF-IR (c) with dilution curves of rat liver (—▲—) and submaxillary (—■—) extracts in the respective radioimmunoassays

tide corresponding to the 901–928 sequence of *RB*-P (19, 34, 35) as standard antigen, ^{125}I -N $^{\alpha}$ -4-hydroxyphenylpropionyl *RB*-P (901–928) as tracer and rabbit anti-synthetic *RB*-P (901–928) serum R0811 (final dilution, $\times 10,500$) (31).

c-K-ras-P The reagents used were a synthetic peptide corresponding to the 166–179 sequence of c-K-ras-P (39) as standard antigen, ^{125}I -c-K-ras-P (166–179) as tracer and rabbit anti-synthetic c-K-ras-P (166–179) serum R4203 (final dilution, $\times 3,500$).

***hst-1/k-FGF*-P** The reagents used were a synthetic peptide corresponding to the 47–80 sequence of *hst-1/k-FGF*-P (56) as standard antigen, ^{125}I -*hst-1/k-FGF*-P (47–80) as tracer and rabbit anti-synthetic *hst-1/k-FGF*-P (47–80) serum R0681 (final dilution, $\times 7,000$).

rat EGF Antiserum SAR301 was obtained from a rabbit immunized with recombinant rat EGF (54) (Earth Chemical, Ako). The reagents used were recombinant rat EGF as standard antigen, ^{125}I -recombinant rat EGF as tracer and rabbit anti-recombinant rat EGF serum SAR301 (final dilution, $\times 140,000$). The tracer was prepared by radioiodinating recombinant rat EGF by the chloramine T method followed by HPLC purification.

RESULTS

Fig. 1 (a, b and c) shows typical standard curves for radioimmunoassays of c-K-ras-P, *hst-1/k-FGF*-P and rat EGF together with serial dilution curves of

rat liver extract in the respective assay systems. The linear dilution curves paralleled the respective standard curves. The detection limits of the assays were as low as 1–95 fmol. Gel filtration profiles of c-K-ras-P-IR and *hst-1/k-FGF*-P-IR in rat liver extract are shown in Fig. 2 (a and b). c-K-ras-P-IR in rat liver extract was eluted as a major single peak of K_{av} 0.35 which was close to that of chymotrypsinogen A of molecular weight 25,000 (K_{av} 0.37) (Fig. 2a). The high peak component showed a band of 21 kDa in SDS-PAGE-immunoblot analysis. The elution profile of *hst-1/k-FGF*-P-IR in gel filtration is shown in Fig. 2b. A main peak of K_{av} 0.37 with small early-eluting peaks were detected and the molecular weight of the main peak component was estimated as 17 kDa and the low early-eluting peak component was 58 kDa by SDS-PAGE-immunoblot analysis. Fig. 3 shows the gel filtration profiles of rEGF-IR in rat submaxillary gland extract (a) and rat liver extract (b). In both cases, rEGF-IR was coeluted with recombinant rat EGF.

Changes in c-K-ras-P-IR, rEGF-IR, *RB*-P-IR and *hst-1/k-FGF*-P-IR levels in regenerating rat liver after partial hepatectomy are shown in Fig. 4. c-K-ras-P-IR concentration was lowered on day 3 ($59.9 \pm 12.1\%$, mean $\pm$ SEM, $P < 0.05$), day 4 ($37.9 \pm 16.4\%$, $P < 0.01$), day 5 ($54.9 \pm 15.0\%$, $P < 0.05$), day 6 ($49.6 \pm 14.5\%$, $P < 0.05$) and day 7 ($58.1 \pm 15.0\%$, $P < 0.05$) of regeneration (Fig. 4a, upper panel). rEGF-IR concentration was markedly raised at 3 h ($237.5 \pm 24.5\%$, $P < 0.01$), 6 h ($191.5 \pm 27.5\%$, $P < 0.05$) and 12 h ($239.3 \pm 36.2\%$,

Fig. 2 Sephacryl S-200 gel filtration elution profiles of c-K-ras-P-IR (a) and hst-1/k-FGF-P-IR (b) in rat liver extract chymotrypsinogen A. MW, $\approx$ 25,000

$P < 0.05$) after resection, and it was then lowered significantly on day 2 ($63.8 \pm 12.0\%$, $P < 0.05$), day 3 ($65.1 \pm 8.8\%$, $P < 0.01$), day 4 ($33.5 \pm 5.9\%$, $P < 0.01$), day 5 ($48.6 \pm 16.4\%$, $P < 0.05$), day 6 ($52.0 \pm 8.1\%$, $P < 0.01$), day 7 ($55.3 \pm 12.0\%$, $P < 0.01$), day 8 ($58.1 \pm 15.0\%$, $P < 0.05$), day 9 ($63.6 \pm 14.2\%$, $P < 0.05$) and day 14 ($69.3 \pm 9.8\%$, $P < 0.05$) of regeneration (Fig. 4b, upper panel). RB-P-IR decreased significantly on day 1 ($53.5 \pm 11.6\%$, $P < 0.01$), day 2 ($74.8 \pm 11.3\%$, $P < 0.05$), day 3 ($75.1 \pm 11.2\%$, $P < 0.05$) and day 4 ($61.0 \pm 10.2\%$, $P < 0.05$) of regeneration (Fig. 4c, upper panel) (31). During 14 days after partial hepatectomy, hst-1/k-FGF-P-IR level in regenerating liver showed no significant changes (Fig. 4d, upper panel).

The effects of gomisins A administration on the changes of c-K-ras-P-IR, rEGF-IR, RB-P-IR and hst-1/k-FGF-P-IR during liver regeneration are summarized in Fig. 4 (each lower panel). Gomisins A exhibited lowering effects on the decreased levels of c-K-ras-P-IR, rEGF-IR and RB-P-IR on the later days of regeneration (Fig. 4, a, b and c, lower panels), while the level of hst-1/k-FGF-P-IR was not affected by gomisins A administration during 14 days after partial hepatectomy (Fig. 4d, lower panel). The decreased level of c-K-ras-P-IR was significantly lowered by gomisins A on day 6 ($51.8 \pm 19.5\%$, $P < 0.05$) and day 7 ($34.9 \pm 17.3\%$,

$P < 0.01$) of regeneration (Fig. 5a, lower panel). rEGF-IR also decreased further on day 7 ($64.7 \pm 6.2\%$, $P < 0.01$) of regeneration (Fig. 4b, lower panel). RB-P-IR level on day 2 and day 9 decreased significantly ($78.6 \pm 6.9\%$, $P < 0.05$, and $61.3 \pm 10.1\%$, $P < 0.05$, respectively) (Fig. 4c, lower panel).

DISCUSSION

Regeneration of rat liver after partial resection has been accepted as a good tool for study of proliferation- and growth-related immediate-early gene expressions in normal cells *in vivo* (10, 16, 18, 21, 22, 25, 29, 37, 53, 55, 58). However, still only limited data have been available regarding to the products of the genes subsequently synthesized. It has been claimed by Fausto and coworkers (17) that one may wonder whether a 2–4-fold increase in the number of transcripts of a few genes observed in early stage of rat liver regeneration leads to a corresponding elevation of their protein products. Diehl and colleagues (13) have observed that levels of both stimulatory and inhibitory GTP-binding proteins correlated poorly with levels of their mRNAs in regenerating rat liver. More studies on gene products, which are evidently essential functioning principles in biochemical events in the cell, are of absolute necessity.

Fig. 3 Sephadex G50 gel filtration elution profiles of rEGF-IR in rat submaxillary gland extract (a) and rat liver extract (b)

In order to quantitate proliferation/growth-related proteins in regenerating rat liver after partial hepatectomy, we have first developed gene protein-specific radioimmunoassays using synthetic peptides and anti-synthetic peptide antibodies. Our previous studies (30, 31) have dealt with proliferation/growth-stimulating gene products, such as *c-myc*-P and *c-fos*-P, and a suppressing gene product, *RB*-P, and we were able to demonstrate regeneration-associated changes in the protein levels in regenerating rat liver by taking advantage of high specificities and sensitivities of the radioimmunoassays used. The products of *c-myc*, *c-fos* and *RB* are all nuclear proteins having DNA binding activity, while *c-ras* gene products (p21) are membrane-associated low molecular weight guanine nucleotide-binding proteins, playing a crucial role in the transduction of external signals in cells (51, 52). The expression of *c-H-ras* and *c-K-ras* has been

reported to coincide with the onset of DNA synthesis during liver regeneration (15, 21, 22). It has been described by Goyette and coworkers (21, 22) that *c-K-ras* and *c-H-ras* transcripts begin to increase at about 18 h after partial hepatectomy in rats, reach a maximum between 24 to 36 h and return to normal level by 72–96 h. The levels of *ras* p21 protein has been found by an immunoprecipitation technique to increase at 48 and 72 h after partial hepatectomy (58). By contrast, an immunocytochemical study (20) using an antibody raised against a synthetic peptide corresponding to the 160–179 sequence of *v-H-ras* (57) has failed to detect immunoreactivity of *c-H-ras* product at any time between 1 and 48 h following partial hepatectomy, although a 2–3-fold increase in the content of *c-H-ras* transcripts was confirmed during the period. The present study used a synthetic peptide corresponding to the 166–179 sequence of *c-K-ras*-P (39) as haptenic immunogen for antibody production and as standard antigen in the radioimmunoassay. The 166–179 sequence is located in the region proximate to the carboxy terminal of the protein and its amino acid compositions are distinctly different from those of the corresponding sequence of *c-H-ras* product. Therefore, the anti-synthetic peptide antibody used in this study was expected to recognize specifically *c-K-ras*-P. The antibody, however, does not differentiate the products of mutated *c-K-ras* genes, since point mutations have been known to occur in positions 12, 13 and 61 of the protein. The present radioimmunoassay for *c-K-ras*-P had a detection limit as low as 100 fmol of the protein. However, we could not observe any significant increase in *c-K-ras*-P-IR in the early stage of regeneration when *c-K-ras* gene expression has been reported to be amplified (22). This may or may not be due to the detection limit of the present radioimmunoassay used. On the other hand, the decreased levels of *c-K-ras*-P-IR were observed after day 3 of regeneration, when the first burst of DNA synthesis and the following mitoses of hepatocytes are known to be almost completed. The kinetics of induction of *c-K-ras* and *c-H-ras* have been described to suggest a role of the products of the genes in mitosis (22). The significantly decreased levels of *c-K-ras*-P-IR on days 3–7 of regeneration may relate to a negative control mechanism of proliferation of hepatocytes after restoration of the residual liver. While *c-K-ras*-P-IR level was reduced, *c-myc*-P-IR and *c-fos*-P-IR were still in elevated levels (30). We have previously suggested (30) that the elevated levels of *c-myc*-P-IR

Fig. 4 Upper panels: Concentration change vs time curves of c-K-ras-P-IR (a), rEGF-IR (b), RB-P-IR (c) and *hst-1/k-FGF-P-IR* (d) in regenerating rat liver after partial hepatectomy. Lower panels: Percent changes by gomisin A administration of c-K-ras-P-IR (a), rEGF-IR (b), RB-P-IR (c) and *hst-1/k-FGF-P-IR* (d) in regenerating rat liver after partial hepatectomy (mean $\pm$ SEM, * P < 0.05, ** P < 0.01).

and c-fos-P-IR in the later stage of rat liver regeneration after partial resection may indicate the implication of the two gene products in DNA replication in nonparenchymal cells in the liver. In this context, there still exists a possibility that an increase in c-K-ras-P, if any, during proliferation of such nonparenchymal cells is masked by suppression of the protein simultaneously occurring to inhibit further proliferation of hepatocytes, and as the result, the c-K-ras-P levels appear to decrease overall.

EGF has been reported to be a potent stimulator of DNA synthesis for hepatocytes in primary culture (38). It has been suggested that the endogenous EGF participates in stimulation of rat liver regeneration in early stage after partial hepatectomy (26, 44, 45). The effects of EGF are exhibited by binding to specific membrane receptors on the liver cell. The amount of EGF receptor mRNA has been shown to increase 2–4 h after partial hepatectomy and decrease below control levels 6–8 h (14, 26). However, EGF receptor mRNA levels in later stages of liver regeneration are controversial; they increased (28, 40) or decreased (2, 25, 27) at 18–24 h after partial hepatectomy. The present study revealed a marked increase in rEGF-IR in regenerating rat liver 3, 6 and 12 h after resection, followed by sustained decreases until day 14 of regeneration. The rEGF-IR in regenerating liver as well as normal liver measured by the rat EGF-specific

radioimmunoassay was confirmed to be indistinguishable from authentic recombinant rat EGF. It was supposed to be of extrahepatic origin, which was internalized with the receptor after binding (27, 33). Although EGF, once internalized, is known to be proteolytically processed, the present immunoassay could recognize rat EGF whole molecules which significantly increased in regenerating liver immediately after partial hepatectomy. The early increase in rEGF-IR seems to reflect the amplified mRNA expression of EGF receptor during the same period of regeneration reported (14, 26). No detailed data have been presented with respect to the kinetics of mRNA expressions of EGF and EGF receptor during the late stages of regeneration after 24 h. The sustained marked decreases in rEGF-IR levels during the period of days 2–14 support, though indirectly, the role of EGF as a stimulus for hepatocyte DNA synthesis occurring within 24 h of liver regeneration after partial hepatectomy (40). EGF has been demonstrated to participate in c-ras gene expression and activation of the gene products in various cell lines (7, 9, 27, 36, 41, 46, 48, 49, 60). The present observation of the concomitant decreases in c-K-ras-P-IR and rEGF-IR during liver regeneration is quite suggestive for further study on the repression mechanism of hepatocyte in liver regeneration.

hst-1/k-FGF gene product (*hst-1/k-FGF-P*) is now known to be a member of the fibroblast

growth factor (FGF) family (1). Sakamoto and coworkers (50) have isolated *hst-1* transforming gene from human stomach cancers and *k-FGF* gene isolated from Kaposi's sarcoma by Delli Bovi and Basilico (11) was then identified with *hst-1* gene (12). The expression of *hst-1* gene has been detected in early mouse embryos (61) and *k-FGF* gene expression has been shown to be detectable during the very first stage of mouse embryonic development (3). However, Northern blot analysis has failed to demonstrate the expression of either *hst-1* or *k-FGF* gene in normal adult tissues. Recombinant *hst-1* protein having molecular weight of 17.5 kDa (42) has been shown to stimulate human umbilical vein endothelial cell growth (43). The product of *k-FGF* has been reported to induce mesoderm formation in *Xenopus laevis* animal pole cells and stimulate DNA synthesis in mammalian fibroblasts (47). We demonstrated, using a specific radioimmunoassay for *hst-1* gene product, in this study that rat liver contains a *hst-1/k-FGF*-related protein of 17 kDa. The 79-171 and 172-206 sequences of *hst-1/k-FGF*-P show significant homology with the sequences of other members of the FGF family in the corresponding regions. We thus selected the 47-80 sequence to synthesize an antigen peptide. The recombinant *hst-1* protein (17.5 kDa) produced in silkworm cells (42) has been shown to have the 59-206 sequence of the predicted structure of *hst-1* gene product based on the cDNA sequence (56). The 17 kDa component of *hst-1/k-FGF*-P-IR recognized by the current anti-synthetic peptide antibody is assumed to correspond to the N-terminal-cleaved form of *hst-1/k-FGF* protein. Among the six proteins examined in our serial studies on proliferation/growth-related proteins in regenerating rat liver after partial hepatectomy (24, 30, 31), *hst-1/k-FGF*-P-IR was the only one that did not show any significant change in liver concentration throughout regeneration. Considering the biological properties so far demonstrated, this observation indicating little participation of *hst-1/k-FGF* protein in rat liver regeneration after partial hepatectomy seems to be convincing.

Our previous study (24) demonstrated that gомisin A has an enlarging effect on hepatocytes in mitotic stage on days 3 and 4 and an enhancing effect on the increase in *c-myc*-P-IR in the following period (days 5-8) in regenerating rat liver after partial hepatectomy. In the present study, the decreases in *c-K-ras*-P-IR and rEGF-IR were shown to be amplified by the drug only during the last stage (days 6 and 7) of regeneration. These suppressive

effects of gомisin A may indicate the existence of a stronger negative control mechanism of the proliferation of hepatocytes growth-promoted by the drug, unless the suppressive effects were not involved in the proliferation of nonparenchymal cells which has been known to occur at this time of regeneration. *RB*-P-IR was also further decreased by gомisin A administration on day 2 and day 9. The suppressive effect of the drug on day 2 of regeneration may relate to the first burst of proliferation/growth of hepatocytes. *RB*-P-IR level was lowered, though not significantly, on days 9 and 10 of regeneration and the suppressive effect of the drug on the decreased levels of *RB*-P-IR observed on day 9 seems to suggest a significant involvement of *RB*-P in rat liver in such a late stage of regeneration. It is, however, difficult to interpret the observation at present, since all the proliferation/growth-related proteins in regenerating rat liver currently examined returned to control levels by day 9 of regeneration after partial hepatectomy.

In summary, we examined quantitatively by radioimmunoassays the changes in *c-K-ras* and *hst-1/k-FGF* gene products and EGF in rat liver during the course of regeneration after partial hepatectomy. *c-K-ras*-P-IR and rEGF-IR as well as *c-myc*-P-IR, *c-fos*-P-IR and *RB*-P-IR previously reported all showed regeneration-associated different patterns of the concentration change vs time curves, indicating clearly the significant participation of *c-K-ras* product and EGF as well as *c-myc*, *c-fos* and *RB* gene products by integrated coordination of the respective unique roles in cell proliferation and growth in regenerating rat liver after partial resection. In addition, the effects of gомisin A on *c-K-ras*-P-IR, rEGF-IR and *RB*-P-IR demonstrated in this study provided additional evidence for the growth-promoting activity of gомisin A on hepatocytes during regeneration of partially resected rat liver which we have suggested in our previous study (24). Although further study is required, gомisin A may be a potential reagent useful for analysis of biochemical events occurring in hepatocyte proliferation/growth processes in regenerating rat liver.

Received 10 January 1995; and accepted 31 January 1995

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