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Expression of ras gene family as result of compensatory renal growth in mice

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Summary. We have examined several gene expressions during the process of compensatory renal growth in mice following unilateral nephrectomy. On the 3rd, 5th and 7th days after operation in young mice (age: 5w), unilateral nephrectomy induced weight gain of the remaining kidney, but not in adults (age: 15w). It also induced maximum expression of c-H-ras and c-K-ras on the 3rd day in both young and adult mice, but there was no increase in N-ras in either. The expression levels of c-H-ras and c-K-ras were higher in young mice than in adults. However no expression of c-myc was detected at any point. Expression of metallothionein-I (MT-I) gene was detected during the first 12 h after unilateral nephrectomy both in the liver and the contralateral kidney. These data suggest that c-H-ras and c-K-ras gene expressions are in some way related to compensatory renal growth in mice and may be strongly related to hyperplasia in the contralateral kidney.

Key words: Compensatory renal growth – ras gene family – Gene expression

Compensatory renal growth (CRG) following hemineph-rectomy is a physiological cell growth processes [17]. The mechanisms which regulate this process have been discussed elsewhere but are still unclear [8]. During the course of similar compensatory proliferation of the liver following partial hepatectomy [4], the transcriptions of c-H-ras, c-K-ras and c-myc are significantly elevated, whereas other proto-oncogenes appear to be unchanged [9, 14]. The regulated expression of these proto-oncogenes suggests that these genes play an important role in the process of liver regenaration. At present, more than 20 oncogenes identified by their association with retroviruses or by DNA transfection have been described [3,6] Proto-oncogenes present in the genome of normal cells are conserved during evolution [3], and are believed to play some essential role in the control of normal growth, differentiation or embryogenesis [3, 5, 6]. Beer et al. demonstrated that there was a lack of change in the abundance of several proto-oncogenes without c-K-ras transcript following unilateral nephrectomy, and that experimental evidence for the expression of cellular oncogenes in CRG is still unclear [2]. However, recent studies by L. T. Norman et al. showed that the time course of mRNA expression within the first 48 h differed in hyperplasia and hypertrophy [16].

On the other hand, several factors are known to modify the hypertrophic response of the remaining kidney [8]. Aging is one of the important factors that influence the pattern of growth, which is predominantly hyperplasia in infant animals and hypertrophy in adults [11–13].

In the present study we observed an elevation of ras proto-oncogene expression after unilateral nephrectomy. These findings may be of help in identifying the regulation mechanism of CRG.

Materials and methods

Animals and RNA extraction

Male ddY mice (purchased from Kyushu Animal Inc., Japan) (age: 5w, 15w) were reared at the Laboratory Animal Center for Biomedical Research, Nagasaki University, School of Medicine. Right unilateral nephrectomy (or sham operation) under nembutal anesthesia was performed in the prone position. Animals were sacrificed at various intervals, from 1 h to 7 days after the operation. The left kidney and liver were excised as quickly as possible and frozen at -70°C. The tissues were weighed and homogenized in guanidine isothiocyanate buffer (4 M guanidine isothiocyanate, 25 mM sodium citrate pH 7.0, 0.5% sarkosyl, 0.1 M beta-mercaptoethanol) and the homogenate was placed on a CsCl cushion (5.7 M CsCl, 0.1 M EDTA pH 7.0). Total RNA was pelleted by centrifugation for 18 h at 70,000 g [10]. The RNA was solubilized in double-distilled water and used for Northern analysis.

Northern analysis

Total RNA (20μg per lane) was separated on 1.0% agarose-2.2 M formaldehyde gels and transferred to nylon membranes (Zeta-Probe, Bio-Rad), followed by baking at 80°C for 2 h and incubation

Table 1. DNA probes of various gene fragments

Classification	Probes			
	Transcript	Fragment	Size	Reference
c-H-ras	1.4 (kb)	6.4 (kb)	BamHI	(1982) Nature 298:343
c-K-ras	5.2 2.0	2.4	EcoRI	(1983) Nucl Acid Res 11:8112
N-ras	3.9 2.4 1.5	0.9	PvuII	(1983) Proc Natl Acad Sci USA
V-myc	2.7	2.9	BamHI	(1983) Proc Natl Acad Sci USA 80:2500
γ-Actin	2.35	0.17	AbaI/BglII	(1983) J Virol 47:611
metallothionein-I	1.7	1.4	DraI/EcoRI	(1984) Proc Natl Acad Sci USA 81:7392

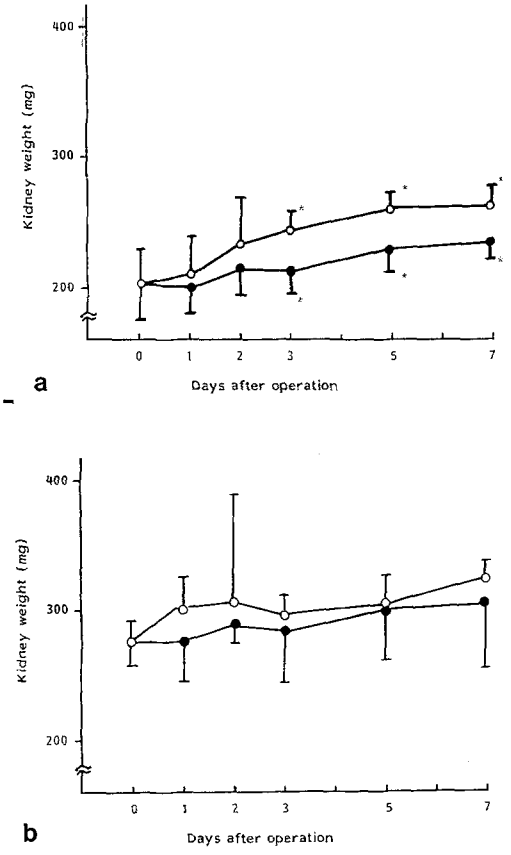

Fig. 1a and b. Kidney weight after unilateral nephrectomy (○: $N = 3$) and sham operation (●: $N = 3$). **a** 5w mice, **b** 15w adult mice. Data represent means ± SE. The two groups were compared using Student's t-test. * $P < 0.05$

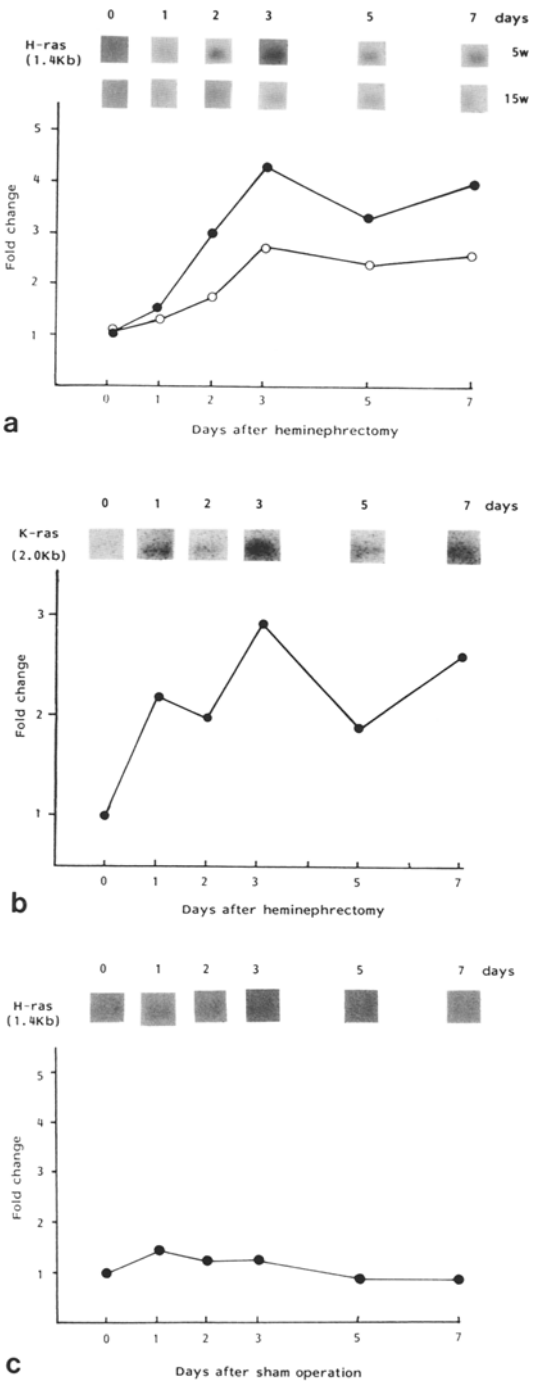

Fig. 2a. Analysis of c-H-ras messenger RNA following unilateral nephrectomy in 5w mice (●: $N = 2$) and 15w mice (○: $N = 2$). **b** Analysis of c-K-ras messenger RNA following unilateral nephrectomy in 5w mice. **c** Analysis of c-H-ras messenger RNA following sham operation in 5w mice. 20 µg of total cellular RNA were isolated from murine kidneys after the operation. After electrophoresis and transfer to nylon membranes, filters were hybridized with ^{32}P-labeled probes. Autoradiograms were obtained and quantified spectrophotometrically. Absorbance values for the normal kidney were arbitrarily assigned a value of 1

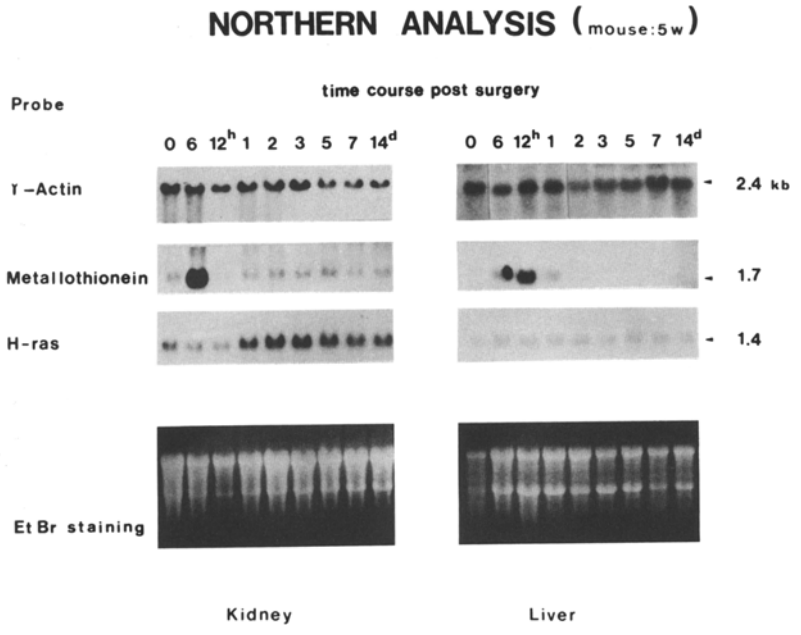

Fig. 3. Liver and contralateral kidney RNA expression during compensatory renal growth. 20 μg of total cellular RNA were isolated from murine kidneys and livers following unilateral nephrectomy in 5w mice. Analysis performed as described in Fig. 2

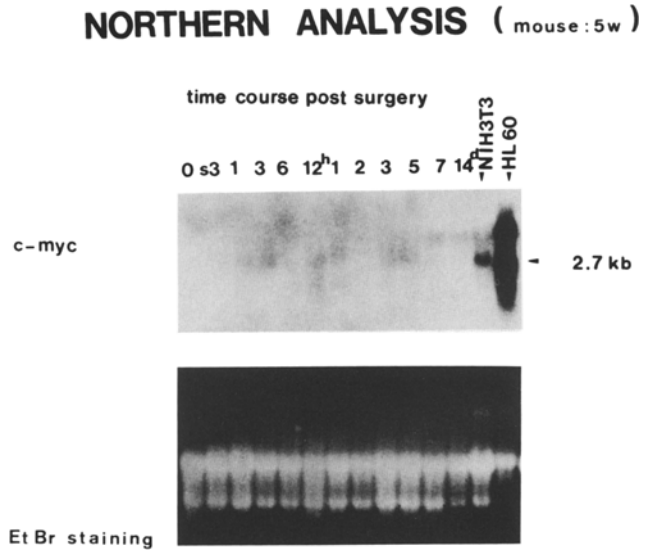

Fig. 4. Analysis of c-myc messenger RNA following unilateral nephrectomy in 5w mice. 20 μg of total cellular RNA were isolated from murine kidneys following unilateral nephrectomy and from NIH3T3. 10 μg of total cellular RNA were isolated from HL-60. Analysis performed as described in Fig. 2. S = sham operation

in prehybridization buffer (50% deionized formamide, 4 × SSPE (1 × SSPE: 10 mM sodium phosphate pH 7.0, 10 mM EDTA, 180 mM NaCl), 1% SDS, 0.5% skim milk, 0.2 mg/ml yeast RNA, 0.5 mg/ml denatured salmon sperm DNA) at 42°C for 16 h.

DNA probes and hybridization

The DNA for probes (Table 1) was labeled using a random prime labeling system (Multiprime kit, Amersham) with [alpha^{32P}]-dCTP (3,000 Ci/mM, Amersham) and denatured. The membranes were then hybridized with probes [2–5 × 10^6 cpm/ml] in hybridization buffer (50% deionized formamide, 3 × SSPE, 10% dextransulfate, 1% SDS, 0.5% skim milk, 0.4 mg/ml yeast RNA) at 42°C for 18 h, and were washed in 0.1 × SSC (1 × SSC; 150 mM NaCl, 15 mM trisodium citrate pH 7.0), 0.1% SDS, at 42°C for 30 min, followed by exposure on an X-ray film (Sakura [new brand name is Konica] type new A) with two intensifying screens at -80°C for 2 days. Autoradiographs were scanned for quantification with an F-808 Cosmo densitometer (Cosmo Co., Ltd.).

Results

Kidney weight after unilateral nephrectomy

In the 5w mice, both the hemi-nephrectomized and sham-operated groups gained kidney weight post-operatively, because of growth. However, from the 3rd to 7th days there was a significant difference between the two groups ($p < 0.05$) (Fig. 1a). In the 15w adult mice, there was no significant kidney weight difference between the hemi-nephrectomized group and the sham-operated group (Fig. 1b).

Gene expression during compensatory renal growth

We determined the size and amount of c-H-ras, c-K-ras, and N-ras transcripts in the total RNA obtained from the mouse kidneys at varying times after heminephrectomy and sham-operation. Gamma-actin gene as a probe was used to qualify and quantify RNA loading. Ethidium bromide staining was also used to quantify RNA loading. In the kidneys of the 5w mice, c-H-ras and c-K-ras transcripts were increased after heminephrectomy. We found a 4 times increased peak in c-H-ras on the third day following heminephrectomy. In contrast to the 5w mice, we found a two times increased peak in the 15w adult mice (Fig. 2a). As expected, a modest increase in c-K-ras message was also seen in the 5w mice (Fig. 2b). However, in the sham-operated mice, these peaks were not induced (Fig. 2c). N-ras transcripts were detected, but only at low levels and no peaks were obvious (data not shown). Expression of MT-I gene was detected during the first 12 h

after unilateral nephrectomy both in the liver and the contralateral kidney. In the liver of both the young and adult mice, c-H-ras transcripts were at basic levels (Fig. 3). The 2.7 kb c-myc transcript was observed in the HL-60 (human promyelocytic leukemia cell line) and NIH3T3. They were positive controls. We could not detect significant expression of c-myc after unilateral nephrectomy (Fig. 4).

Discussion

Following unilateral nephrectomy, the contralateral kidney cells are increasingly sensitized by the circulation and/or local growth factors to act as mitogens and/or hypertrophic reagents [1, 5, 17, 18]. We have examined the weight of the remaining kidney following unilateral nephrectomy. Among the young mice, the heminephrectomized group showed a greater kidney weight gain on the 3rd, 5th, and 7th postoperative days than the sham-operated group. The kidney weight of the heminephrectomized adult mice was also greater than that of the sham-operated adults, but there was little or no difference from the control group. These data suggest that the pattern of remaining kidney growth in young mice differs from that in adults.

In the present study, we examined the transcripts of several genes during the process of compensatory growth in the murine kidney following unilateral nephrectomy. In young mice, we observed peaks in c-H-ras and c-K-ras proto-oncogene expressions on the third post-operative day, at which time the peak was 4 times for c-H-ras and two times for c-K-ras. This time specific expression pattern may have some relation to compensatory renal growth, because there is a report that the DNA content of the remaining kidney is elevated at 24 h, especially in young animals [2]. However, in what way this expression is related to mitogenic reaction and how it is regulated are still unclear. R. Karp et al. emphasized that the infant was capable of generating new DNA (hyperplasia), while the adult was almost completely unable to do so (hypertrophy) [11]. L. T. Norman et al. demonstrated that c-H-ras expression was not detected after unilateral nephrectomy in adult rabbits [16]. The c-H-ras and c-K-ras oncogene expression may be more related to hyperplasia in the contralateral kidney. And we could not detect c-myc transcript activation in CRG. M. Goyette et al. demonstrated that c-myc expression increased markedly during liver regeneration [9]. Perhaps the reason is that there is less DNA synthesis in CRG than in liver regeneration [4, 8].

Another point was that both young and adult c-H-ras messenger RNA levels were lower in the liver than in the contralateral kidney regardless of elapsed time. Expression of MT-I gene is induced by surgical trauma, and MTs are synthesized in the liver and kidney [7]. We have found that expression of MT-I gene is detected during the first 12 h after unilateral nephrectomy both in the liver and the contralateral kidney. The response to a reduction in renal mass may be an event specific to the contralateral kidney.

In conclusion, we have found that aging is one of the important factors influencing the growth process of cells. This study suggests that the difference of c-H-ras and MT-I gene expression during CRG may shed some light on organ specific signals after unilateral nephrectomy. Though one goal of these studies was to identify the regulation mechanism of compensatory renal growth, more study is needed to be made in order to define how the transcripts of c-H-ras and c-K-ras are related to compensatory renal growth.

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