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DIFFERENTIAL EXPRESSION OF THE MURINE AND RAT RENIN GENES IN PERIPHERAL SUBCUTANEOUS TISSUE

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We have previously identified peripheral subcutaneous tissue as a bonafide site of primary renin expression in the mouse fetus by virtue of oncogene mediated tumorigenesis in transgenic mice. In this report we demonstrate that the murine renin genes are differentially expressed in this tissue. Through selective breeding and differential primer extension we demonstrate that Ren-1^d and Ren-1^c transcripts were several fold more abundant than Ren-2. Renin transcripts were also identified in fetal subcutaneous tissues of Spontaneously Hypertensive (SHR) and Wistar Kyoto (WKY) rats. We conclude from these studies that expression of renin during fetal development may be widespread in rodents with its temporal and spatial localization consistent with a role in fetal development. © 1990 Academic Press, Inc.

The renin genes of inbred mice are commonly identified by the nomenclature Ren-1^c, for the Ren-1 allele in strains of mice containing a single renin gene, and Ren-1^d and Ren-2, for the Ren-1 allele and the duplicated locus, respectively, in strains of mice containing two renin genes. Although each gene contributes equally to transcripts present in the kidney (1), the classical site of renin synthesis, each gene exhibits a complicated pattern of differential expression at a number of extra-renal sites (1-4).

We have previously described the construction of transgenic mice containing a transgene consisting of a renin regulatory region fused to the SV40 T antigen oncogene (5,6). Expression of the transgene mRNA and the resultant oncoprotein was shown to be expressed in a tissue- and cell-specific manner appropriate for a renin gene (6). These transgenic mice developed neoplasms in the subcutaneous tissue, the RNA from which was shown to contain renin mRNA (5). Furthermore, renin transcripts were shown by northern hybridization in fetal subcutaneous tissue from non-transgenic mice identifying this tissue as a site of primary renin expression during normal mouse development.

The differential expression of the mouse renin genes in many extra-renal tissues (1-4) prompted us to examine the relative contribution of each of the mouse renin genes in subcutaneous tissue. We also examined whether this expression pattern was unique to mouse or was also present in rat. Herein we demonstrate the differential expression of the murine renin genes in subcutaneous tissue and the presence of renin transcripts in subcutaneous tissue of fetal rats.

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MATERIALS & METHODS

Production and Analysis of Transgenic mice: Construction of the renin promoter-T antigen transgene and production of transgenic mice has been previously described (5,6). Transgenic mice were constructed on a genetic background containing the Ren-1^c gene. In order to obtain transgenic mice on a genetic background containing Ren-1^d and Ren-2, the Ren-1^c transgenic mice were bred to DBA/2J mice for two successive generations. The genetic constitution at the renin locus of the resultant mice was examined by Southern blot analysis. A PvuII restriction fragment length polymorphism (RFLP) was used to differentiate between the three genes as previously described (7). DNA was isolated from tail biopsies and Southern blots were performed as previously described (8,9). A Ren-2 cDNA clone (10) which detects all three renin genes was used as a probe.

RNA was purified from tumor tissues as described (11). Differential primer extension reactions were performed to differentiate between Ren-1 and Ren-2 transcripts as previously described (1,5).

Analysis of Renin mRNA in Fetal Rats: Staged pregnant SHR and WKY rats were obtained from Harlan Sprague-Dawley. Pregnancies were terminated at 15.5 and 18.5 days post coitum. Individual fetuses were decapitated, completely eviscerated and washed to remove contaminating viscera. RNA was purified from the resultant carcasses as previously described (5). Northern blots containing 100 μg of total RNA were probed with a human renin cDNA which hybridizes to mouse, rat and human renin mRNAs (data not shown). Correctly sized messages were identified by co-migration with kidney RNA from a CD-1 rat.

RESULTS

In order to determine if the mouse renin genes are differentially expressed in subcutaneous tissue, transgenic mice containing only the Ren-1^c gene were bred for two successive generations to DBA/2Ros mice containing the Ren-1^d and Ren-2 genes. The mice from the first generation were all F_1 with respect to the renin loci on chromosome 1. However, these chromosomes randomly assorted in the second backcross resulting in two populations of mice with respect to chromosome 1, those containing either all three genes each in a single copy (which were not examined since our primer extension assay could not differentiate between the Ren-1 alleles), or containing only the DBA renin genes each in two copies (Table 1).

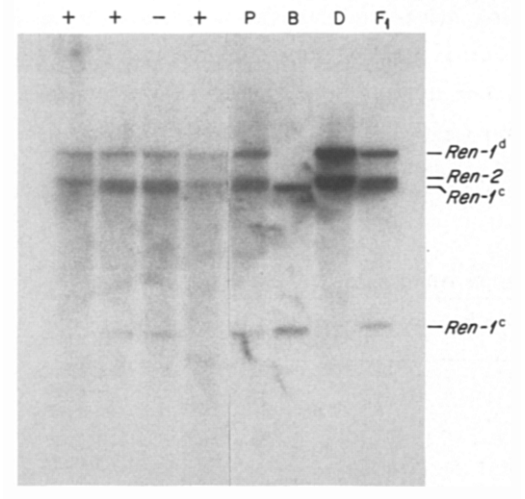
TABLE 1
SUMMARY OF MICE USED IN THIS STUDY

MOUSE	Tg	RENIN GENOTYPE	SUBCUTANEOUS* TUMORS EXAMINED
		Tg(BCF) X BCF	
820/1	+	Ren-1^c	Tumor E
		Tg(BCF) x DBA/2ROS (FIRST BACKCROSS)	
1641/1	+	Ren-1^c,Ren-1^d,Ren-2	N/A
		1641/1 X DBA/2ROS (SECOND BACKCROSS)	
1836/1	+	Ren-1^c,Ren-1^d,Ren-2	N/A
1836/2	-	Ren-1^c,Ren-1^d,Ren-2	NO TUMORS
1848/1	+	Ren-1^d,Ren-2	Tumors A,B,C
1848/2	+	Ren-1^c,Ren-1^d,Ren-2	N/A
1848/3	-	Ren-1^c,Ren-1^d,Ren-2	NO TUMORS
1849/1	+	Ren-1^d,Ren-2	Tumor D
1849/2	-	Ren-1^d,Ren-2	NO TUMORS

*same tumor nomenclature is used in Figure 2.
N/A, tumors were not assayed, incorrect genotype.

Mice from the second backcross were examined for their renin gene constitution by Southern blot analysis of DNA isolated from tail biopsies. Figure 1 shows the results of this analysis. The identity of the specific bands are given and can be compared to the control lanes containing only Ren-1^c (B), both Ren-1^d and Ren-2 (D) or all three genes (F_1). The parental F_1 mouse from the first generation backcross (P) contains all three renin genes as expected. Four offspring from the second backcross are shown in Figure 1. Since the transgene is integrated into a single chromosomal site per diploid genome only 50% of the offspring will be transgenic. The presence of the transgene was assayed separately by dot blot hybridization using the transgene as a probe (data not shown). In addition, the probability of achieving a genetic constitution homozygous for the DBA renin alleles in the second backcross is 50%. Therefore, since the transgene is not integrated into chromosome 1, the overall frequency of obtaining transgenic mice on the desired genetic background is 25%. As summarized in Table 1, two transgenic mice containing Ren-1^d and Ren-2 but not Ren-1^c (1848/1 and 1849/1) were obtained from a total of seven second backcross mice.

The level of renin mRNA in eviscerated fetuses is below the threshold of detection by our primer extension assay making a direct assessment of the ratio of Ren-1 to Ren-2 transcripts difficult. Therefore, we chose to examine the T antigen-induced subcutaneous tumors which arose in adult mice. These tumors are clonally derived and therefore typify the expression pattern of a single cell. Four subcutaneous tumors (A-D) were recovered from two transgenic mice



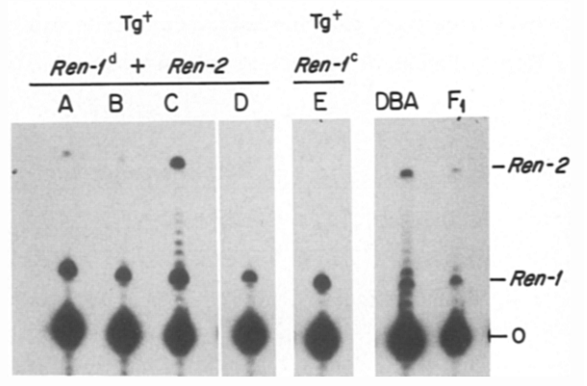


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Figure 1. Southern blot analysis of backcrossed transgenic mice.
DNA was isolated from tail biopsies, digested with PvuII and electrophoresed through 0.8% agarose. The blot was probed with a Ren-2 cDNA. Different renin genes and Ren-1 alleles were identified on the basis of size as previously described (7). +, transgenics; -, negative littermates; P, parental F_1 transgenic; B, C57BL/6 which contains only Ren-1^c; D, DBA/2Ros which contains Ren-1^d and Ren-2; F_1, C57BL/6 X DBA/2Ros mice. Transgenic mice were identified by dot blot hybridization using the transgene as a probe (data not shown).

Figure 2. Primer extension of subcutaneous tumor RNA from backcrossed transgenic mice.
RNA was isolated from subcutaneous tumors and subjected to differential primer extension to assess the relative contribution of Ren-1 and Ren-2 transcripts. A-D, four independent subcutaneous tumors from transgenic mice (see Table 1). E, subcutaneous tumor from a transgenic mice containing only Ren-1^c (see Table 1). DBA, DBA/2Ros kidney RNA; F_1, kidney RNA from C57BL/6 X DBA/2Ros F_1 mice.

containing the DBA/2Ros renin alleles (Table 1) all of which expressed renin and contained correctly processed full length renin transcripts (data not shown). Our breeding scheme allowed us to fix the Ren-1^d allele (and the Ren-2 gene) so that differential primer extension could be applied to examine the relative contribution of the renin loci to the renin transcripts within the tumor samples. Figure 2 shows the results of this primer extension analysis. The control kidney sample from DBA mice or F_1 mice contains both species of renin transcripts. As expected the relative proportion of Ren-2 transcripts is less in the F_1 mice since all three renin genes are equally expressed in the kidney and the Ren-1 band is a mixture of both Ren-1 alleles. The RNA from the subcutaneous tumor (tumor E) arising in mice containing only the Ren-1^c gene (820/1, Table 1) contained only the Ren-1 band demonstrating the fidelity of our assay. An analysis of subcutaneous tumors A-D (Table 1) revealed the presence of both Ren-1 and Ren-2 transcripts. However, the level of Ren-1 transcripts was in great excess (10 fold) over Ren-2 transcripts in 3 of the 4 samples. In the other sample, the Ren-1 transcripts were approximately 3-fold over represented as compared to the Ren-2 transcripts. These results suggest that Ren-1^d is expressed from 3- to 10-fold greater than Ren-2 in subcutaneous tissue. That one tumor sample contained relatively higher Ren-2 levels suggests some heterogeneity in the population of renin expressing cells in subcutaneous tissue. The level of total renin transcripts in tumors from mice containing Ren-1^d and Ren-2 was roughly equal to tumors containing only Ren-1^c (Figure 2, and data not shown) suggesting that both Ren-1^d and Ren-1^c are equivalently expressed.

With the identification of renin transcripts in fetal subcutaneous tissue it became important to address whether this finding was unique to the mouse. Therefore, fetal rats were obtained from timed pregnancies and were sacrificed at 15.5 and 18.5 days post coitum. SHR and WKY fetal rats were obtained at both stages of development and eviscerated as previously described (5). RNA was purified from the resultant carcasses and was analyzed by northern blot hybridization. As shown in Figure 3, renin transcripts were easily detected in the control kidney samples but not in 100 μg of liver or submandibular gland RNA consistent with the tropism of the rat renin gene and demonstrating the accuracy of our northern blots. Renin transcripts as identified by co-

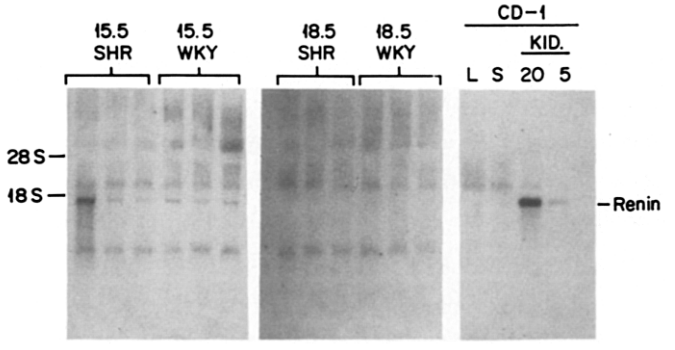

Figure 3. Renin mRNA in fetal rat subcutaneous tissue.
100 μg of RNA from eviscerated rat fetuses at two developmental stages was assayed for renin expression as described in Materials and Methods. L, 100 μg rat liver RNA; S, 100 μg rat submandibular gland RNA. Kid., rat kidney RNA in the indicated amounts (20 and 5 μg). Fetal tissues were obtained from SHR and WKY rats, and adult tissues were obtained from a CD-1 rat.

migration with the control kidney transcripts were clearly visible in the 15.5 days post coitum carcasses but not in the 18.5 days post coitum carcasses. This result confirms our previous observation of renin mRNA in fetal mouse carcasses. However, it appears that the mouse and rat renin genes may follow slightly different temporal expression patterns since transcripts were still detected in carcasses of newborn mice (5).

DISCUSSION

The presence of renin transcripts in the subcutaneous tissues of the mouse has recently become evident through specifically targeted oncogene mediated tumorigenesis in transgenic mice (5). Mice containing a transgene consisting of 4.6 Kb of the Ren-2 5' flanking region, which acts as a tissue and cell-specific regulatory element (6), fused to SV40 T antigen develop tumors derived from subcutaneous tissue. Since these tumors contained renin transcripts it was suggested that they arose from a population of renin expressing cells in that tissue (5). Here we report that like many other extra-renal tissues, the mouse renin genes are differentially expressed in subcutaneous tissue.

The temporal and spatial expression of renin in fetal mouse and rat subcutaneous tissue is similar to the appearance of angiotensin II receptors in this tissue (12,13). Both renin mRNA and the concentration of angiotensin II receptors markedly decrease near or just after birth. In addition, angiotensinogen mRNA has been identified in brown fat and mesentery (14) suggesting that several components of the renin angiotensin system may be closely physically localized. It will be interesting to determine whether the tumor cells themselves or the cells from which they derive contain angiotensin II receptors.

Our ability to detect renin mRNA in the subcutaneous tumors in adult transgenics by oncogene mediated tumorigenesis suggests that a small population of renin expressing cells may still be present in the mature tissue. Accordingly, it is possible that the decrease in renin mRNA levels observed after birth in the mouse and between 15.5 and 18.5 days post coitum in the rat reflects a decrease in the proportion of renin expressing cells due to growth of the surrounding tissue as opposed to a decrease in renin transcription on a per cell basis. However, we cannot rule out the possibility that the tumors formed in these mice as a result of re-expression of renin (and the transgene) in a cell lineage which expressed renin earlier in development but has temporally inactivated its transcription. This could occur as a result of some somatic mutation, the nature of which is unclear. If the latter interpretation is correct, then such a mutation should not exhert its effects in cis, since multiple renin genes from independent loci can be expressed in a single tumor sample (Figure 2 and ref. 5). Therefore, the mutational inactivation of a repressor molecule or activation of a positive transcription factor may be likely candidates.

Although our results strongly suggest that renin is expressed (at least in fetal development) in the subcutaneous tissues of the mouse and rat, there are heretofore no reports demonstrating direct evidence for the same expression profile in humans. Nevertheless, there are at least two reports in which renin secreting "ectopic" tumors have been identified in subcutaneous tissue or in other soft tissues (15,16). The former was identified when a patient presented hypertension

which was treated by surgical excision of the tumor tissue. It remains possible that these tumors were derived from cells in which renin expression is a normal or potential occurrence and not strictly ectopic.

It should be noted that additional tissues have recently been reported and added to the repertoire of renin expressing tissues, including the mouse anterior prostate (3), subcutaneous tissue (5), and the mouse and rat liver, brain, hypothalamus, spleen, thymus, and lung (2). Nevertheless, any further consideration of renin and the renin-angiotensin system in development or the normal functioning of subcutaneous tissue will require further experimentation.

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