Proto-Oncogene of Genomic DNA, Related to the Human Epidermal Growth Factor Receptor (EGFR) Gene, from Clinically Normal Domestic Animals

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ABSTRACT. Genomic DNAs of cattle, horses, pigs, dogs, cats and chickens were surveyed using Southern blot hybridization analysis, with a human EGFR cDNA fragment. Several bands with different numbers and molecular weights were observed under the condition of low stringency in the individual animal species. The bands showing DNA polymorphism were observed among bovine genomic *Pst*I-digested DNAs from 4 individuals and *Eco*RI-digested genomic DNAs from 4 chickens. These results may provide basic data which are useful for analysis of tumorigenetic mechanisms in domestic animals. —KEY WORDS: epidermal growth factor receptor gene, hybridization, proto-oncogene.

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Human epidermal growth factor receptor (EGFR) has been demonstrated to be homologous with the avian erythroblastosis virus oncogene product, v-*erb*B [1] and it has been suggested that this product is a truncated form of the EGFR, which is produced from the host cellular proto-oncogene, c-*erb*B [6]. The EGFR consists of two separated domains; an external amino portion, which is glycosylated and binds the epidermal growth factor, and a cytoplasmic carboxylated portion, which contains EGF-dependent protein kinase activity capable of autophosphorylation and other substrates at specific tyrosine residues. The EGF-stimulated regulatory system may play a role in carcinogenesis as elevated levels of the EGFR gene have been demonstrated in tumors of the breast and stomach [5] and the rearrangement of the EGFR gene has been demonstrated in some human brain tumors [3].

Proto-oncogenes of genomic DNA have already been reported Southern blot analysis of 7 oncogene probes (c-*myc*, c-*yes*-1, c-*erb*B -2, c-*ros*-1, v-ki-*ras*, v-Ha-*ras*, and v-*myc*) in clinically normal animals of various species [4]. With the view of studying tumorigenesis mechanisms in domestic animals, we surveyed EGFR genes in clinically normal animals of various species using Southern-blot hybridization analysis [7].

Testes were surgically removed from 2 males in each species of bovine (Japanese Black), horse (Thoroughbred), pig (Landrace White), dog (mongrel) and cat (Japanese domestic). Blood cells were taken from 2 chickens (White Leghorn). The human placenta was used as a control. All tissues were stored at −70°C until required for use. High molecular-weight genomic DNAs were isolated from 1–1.5 g of the tissue specimens [4]. The 10 μg genomic DNA samples were digested with restriction endonucleases (*Bam*HI, *Eco*RI, *Pst*I or *Hin*dIII) (Takara Shuzo Co., Ltd., Kyoto, Japan). These digested DNAs were subjected to electrophoresis on 0.8% w/v agarose gel and transferred to nylon membrane filters (Magnagraph nylon, Funakoshi Co., Ltd., Tokyo, Japan) as described [2]. The high molecular weight DNAs on the filters were hybridized with DNA fragments of the human EGFR cDNA *Cla*I-*Cla*I 2.4 kilobase pairs [11] and human c-*yes*-1 oncogene *Hin*d III-*Eco* RI 0.55 kilobase pairs [10]. The fragment of EGFR was prepared from a plasmid pE7 that was supplied by the Japanese Cancer Research Resources Bank, Tokyo, Japan, and the fragment of C-*yes*-1 was a gift from Dr. M. Shibata, the Department of Genetics, Institute of Medical Science, the University of Tokyo, Tokyo, Japan. The DNA probes were labeled with [alpha-^{32}P]-deoxycytidine-5'-triphosphate (~111 TBq/mM, ICN Biomedicals Inc., California, U.S.A.) as described in the previous report [4].

The human EGFR gene fragment was hybridized with genomic DNAs from various species of domestic animals under the condition of low stringency (Fig. 1). Strong and weak signal bands were detected by autoradiography in different locations in the individual animal species. The molecular weights of the strong signal bands were shown in Table 1. Homozoic differences were found in bovine genomic *pst* I-digested DNAs and chicken genomic *Eco*RI-digested DNAs (Table 1). To analyze this finding in detail, DNA specimens from further 2 cattle and 2

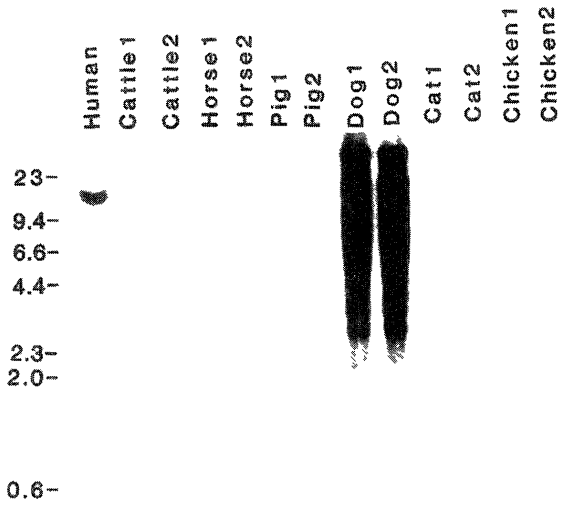

Fig. 1. Southern blot hybridization analysis of genomic DNAs digested with *Hind*III and hybridized under the condition of low stringency. The numbers on the left side refer to the lambda phage DNA digested with *Hind* III used as a size marker; the unit is Kilobase pair.

Table 1. Molecular weight (in kilobase pairs) of DNA restriction fragment homologous to human EGFR gene in domestic animals

Enzymes[a)]	Human	Cattle	Horse	Pig	Dog	Cat	Chicken
*Hind*III	12.0	9.6	15.0	9.6	14.0	6.8	9.9
	4.7	6.0	11.0	4.5	6.8	5.8	8.4
	2.6	3.3	9.0	3.3	3.8	3.7	5.3
		3.0	6.0	2.1	2.6	2.8	4.7
			3.3			2.0	4.4
			2.6				
*Pst*I	9.2	5.8*	6.0	4.6	6.2	6.8	2.9
	2.5	4.4*	2.6	4.3	4.8	5.0	2.5
	2.4	1.9		2.4	2.2	4.5	2.4
	2.1	1.1		2.3	1.9	2.3	2.3
						1.8	
*Eco*RI	7.8	16.0	9.4	9.4	7.7	4.6	10.5*
	6.4	6.2	4.8	2.9	6.3	4.0	5.9
	5.2	4.7	3.3		3.6	3.8	4.6
	3.4	2.4	1.4		3.2	2.3	3.8
	2.2	2.0			2.1	1.1	2.7
	1.6				2.0		
*Bam*HI	18.0	8.8	7.4	3.5	9.8	6.7	7.2
	8.6		4.6	1.5	4.4	1.5	6.7
	5.8		3.5		2.7		3.5
	4.0		2.9		1.9		1.6
	3.4		1.5		1.5		
	1.7						

a) enzyme=restriction endonuclease.
* Bands reveal interindividual differences.

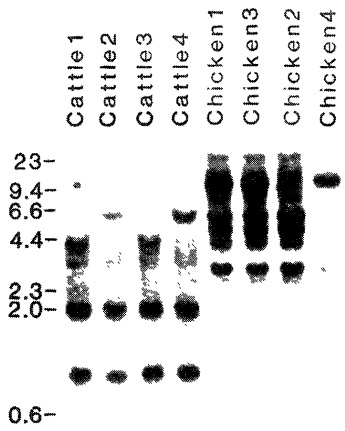

Fig. 2. Southern blot hybridization analysis of genomic DNAs obtained from 4 cattle (*Eco*RI digests) and 4 chickens (*Pst*I digests). Homozoic differences are demonstrated at approximately 5.8 and 4.4 kilobase pairs in the cattle, and 10.5 kilobase pairs in the chickens.

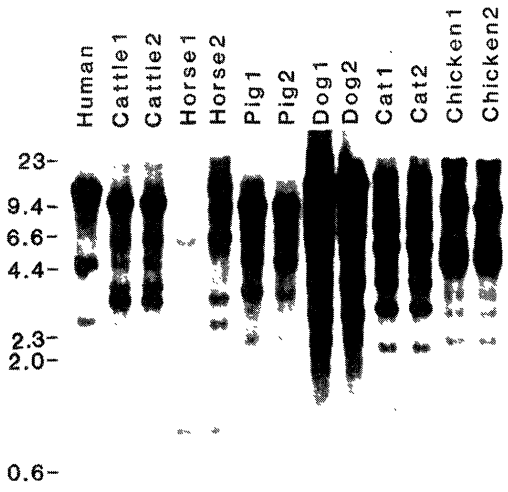

Fig. 3. Southern blot hybridization analysis of genomic DNAs digested with *Hind*III and hybridized under the condition of moderate stringency. Strong signal bands are detected only in dogs.

chickens were reexamined, and differences among individuals were confirmed in both species (Fig. 2). On the other hand, homozoic differences were not observed in the canine genomic DNAs from the 2 and further 8 dogs (data not shown).

The genomic DNAs of each animal were also examined under the condition of moderate stringency. Several strong signal bands were observed only in canine genomic DNAs (Fig. 3). The same filters were rehybridized with human c-*yes*-1 oncogene probe, and no partial digestion was detected in all of the genomic DNA digests.

This study provides evidence that homologous proto-oncogenes to the human EGFR gene exist in all of the domestic animals. The detected bands under the condition of low stringency differed among the species in their numbers and molecular weights. In the canine specimens, the strong signal bands were detected in both conditions of low and moderate stringency. The EGFR cDNA clone used as a probe has encoded all of the 3 domains (extracellular, transmembrane and tyrosine kinase) [9].These detected bands may include several sequences of the other tyrosine kinase family genes or non-specific sequences, homologous to this probe. Accordingly, a shorter fragment of this EGF cDNA clone, e.g. tyrosine kinase domain, must be used as the probe for Southern blot analysis of animal genomic DNAs.

Homozoic differences in the numbers and molecular weights of the bands were demonstrated in the bovine *Pst* I-digested genomic DNAs and chicken *Eco* RI-digested genomic DNAs. An animal has at least one of 2 different homogeneous alleles. In the present study, homozoic difference was not detected in the other restriction endonuclease digests of the cattle and chickens. Consequently, the homozoic differences are attributable to DNA polymorphism. To confirm the hypothesis, a large number of samples must be analyzed.

The analysis of animal proto-oncogenes related to the human EGFR gene may provide basic information which helps to elucidate tumorigenetic mechanisms connected with EGFR in domestic animals.

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