Physical and Radiation Hybrid Mapping of Porcine Tissue Kallikreins

S.C. Fernando, R.D. Geisert, and U. DeSilva

Story in Brief

Kallikreins are members of a multigene family of serine proteases that are widespread throughout living organisms. They have a diverse tissue specific expression pattern and are known to play a significant role in fertilization, digestion, regulation of blood flow, blood coagulation, inflammatory responses, endothelial cell migration, tissue remodeling, tumor-cell invasion and programmed cell death as well as disease related specialized enzymatic activities. To identify and characterize the kallikrein gene family in the pig, and to gain insight about the structure and evolutionary origins of the kallikrein gene region in the pig, we have constructed a BAC (Bacterial Artificial Chromosome) clones –based physical map of the greater kallikrein gene region of the pig genome. We have also mapped the kallikrein gene region to porcine chromosome 6q12-q21.

Key Words: Kallikrein, Early Embryonic Loss, Physical Mapping, RH Mapping, Pig, Genome

Introduction

One of the major goals of the swine industry today is increasing the litter size by overcoming embryonic loss during the first 2 to 3 weeks of pregnancy. In litter-bearing species such as pigs, the conceptus survival depends on the growth and establishment of a placental-uterine interface, which provides adequate vascular blood flow for transport of nutrients throughout the gestation period. Various members of the kallikrein serine protease family are known to release bradykinin through the proteolytic cleavage of kininogen (Bhoola et al., 1992). This release of kinins through tissue kallikrein synthesis has recently received increased attention in therapeutic angiogenesis (Emanueli et al., 2001) with indication that kinins induce increased microvascular permeability and vascular growth (Fabre et al., 1999). This suggests that the Kallikrein-Kininogen-Kinin system could play a role in uterine and placental angiogenesis essential for embryonic and fetal survival in the pig. This led us to investigate the kallikrein gene family in the pig.

Kallikreins are members of a multigene family of serine proteases that are widespread throughout living organisms (Gan et al., 2000). They are found in diverse tissue specific patterns and are known to have highly diverse physiological functions such as fertilization, digestion, regulation of blood flow, blood coagulation, inflammatory responses, endothelial cell migration, tissue remodeling (Yousef et al., 2003); tumor-cell invasion and programmed cell death as well as disease related specialized enzymatic activities (Southardsmith et al., 1994; Gan et al., 2000; Yousef et al., 2003). Currently, extensive research is being carried out on the human kallikrein gene family which shows potential as candidate biomarkers for tumors and human cancers.

Except for kallikrein 4 (isolated from pig dentine, Genbank accession # U76256) no other porcine kallikreins have been identified thus far. As a first step towards isolation, characterization and functional analysis of the porcine kallikrein gene family, we have constructed a BAC clone-based physical map of the greater kallikrein gene region of the pig and mapped the porcine kallikrein region to chromosome 6.

Materials and Methods

Construction of the Physical Map.

40 bp long overgo probes were designed for all known human kallikrein genes using sequence information available in public databases. ^{32}P labeled probes were pooled and used to screen high-density filters of a porcine BAC library with 6.3X coverage of the genome (Pieter de Jong; http://www.chori.org/bacpac/). Clones isolated from the initial screening were further evaluated by probing with individually labeled probes. Insert ends of the BAC clones were isolated by Vectorette PCR end rescue approach (Sambrook and Russel, 2001). Isolated BAC ends were sequence analyzed and BAC-end specific PCR assays were used to screen isolated BAC clones to construct the physical map.

Radiation Hybrid (RH) Mapping.

Radiation hybrid mapping was performed using INRA-Minnesota 7000 rads radiation hybrid panel (IMpRH), which consisted of 118 hamster-porcine hybrid cell lines (Hawken et al., 1999). Primers were developed for pig kallikrein 4 sequence (GenBank accession # U76256) and the entire RH panel was scored by PCR using INRA protocols (http://www.toulouse.inra.fr/lgc/lgc.htm). No PCR product was obtained for the rodent genomic DNA. Data analysis was performed using software available at IMpRH database (http://imprh.toulouse.inra.fr) for chromosome assignment.

Results and discussion

Physical Mapping of the Kallikrein Region.

The screening of high density porcine BAC library with overgo probes designed using all known human kallikreins resulted in 8 BAC clones that contained putative kallikrein gene fragments. Subsequent sequence analysis of BAC insert ends and overgo-based hybridization experiments with human kallikreins resulted in a BAC-based physical map of the porcine kallikrein region (see Fig1).

The assembled contig of the pig kallikrein region containing 8 BAC clones provides ordering information for 12 STSs (Sequence Tagged Site). Of the 12 STSs, 7 correspond to BAC insert ends, 5 to novel kallikrein genes that we identified and 1 to the previously identified pig kallikrein 4 gene. Overlap of clones shown in the contig on Figure 1 was further confirmed by restriction enzyme digest-based BAC fingerprint analysis (Marra et al., 1997). BAC fingerprint analysis also revealed that the total length of the contig to be ~375 kb. We estimate the average distance between STSs in the map to be ~ 28.8 kb.

Based on the physical map and repeated hybridization studies, we have identified clone 069G11 (Fig. 1) to contain all members of kallikrein genes identified by us so far. Systematic and complete sequence analysis of this clone is underway.

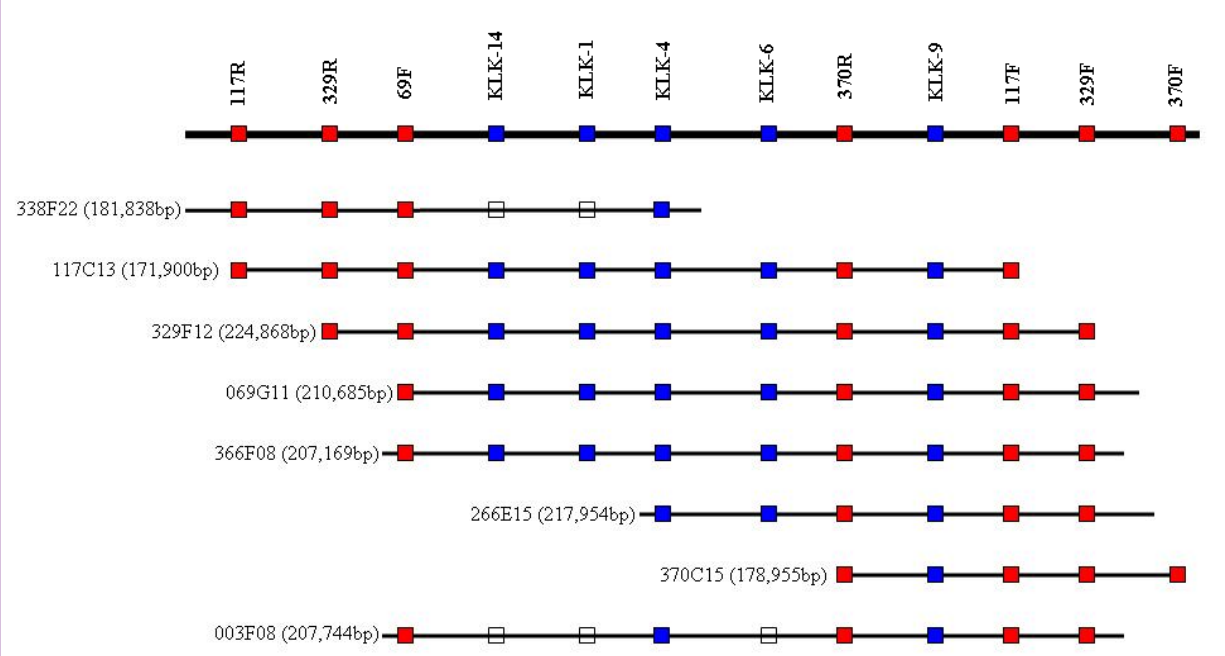

Fig. 1 BAC clone based physical map of the kallikrein gene region. The positions of the STSs are depicted along the top, the clones are shown as horizontal lines. The STSs were developed from clone insert ends (Red), Kallikrein genes (Blue). The unfilled squares on clones 338F22 and 003F08 show STSs not analyzed.

Radiation Hybrid (RH) Mapping

Based on the Human–Pig comparative map (Fronocke et al., 1996) we assumed that the pig kallikrein region may map to porcine chromosome 6. We used a PCR assay developed for porcine kallikrein 4 (KLK4) and a pig-hamster radiation hybrid (RH) panel to map the kallikrein region in the porcine genome. The results revealed that KLK4 marker was linked to first-generation markers of *Sus-scrofa* Chromosome 6 (SSC6) (Hawken et al., 1999) with LOD scores of greater than 6 by two point analysis confirming that the pig kallikrein region is clustered within pig chromosome 6 (see figure 2).

This indicates that kallikrein gene region has conserved synteny between human chromosome 19 (HSA19) and porcine chromosome 6 (SSC 6), and is consistent with the comparative mapping results obtained by bi-directional chromosome painting (Goureau et al., 1996). We have so far used only one marker to map the kallikrein gene region in the RH panel resulting in the fairly low resolution. We are in the process of adding more kallikrein markers to the panel as sequencing results become available.

The physical map generated in this study would be an invaluable resource in further understanding of the role of kallikreins in the pig. The resulting mapping and sequencing data

generated for the porcine greater kallikrein gene region should also provide insight into the evolutionary origins of this structurally complex genomic region. These novel kallikrein genes identified will help us to evaluate the expression of tissue kallikreins in the porcine endometrium and conceptus during the estrus cycle and pregnancy to help us better understand the role of the kininogen-kallikrein-kinin system in placental development and embryonic survival throughout gestation in the pig.

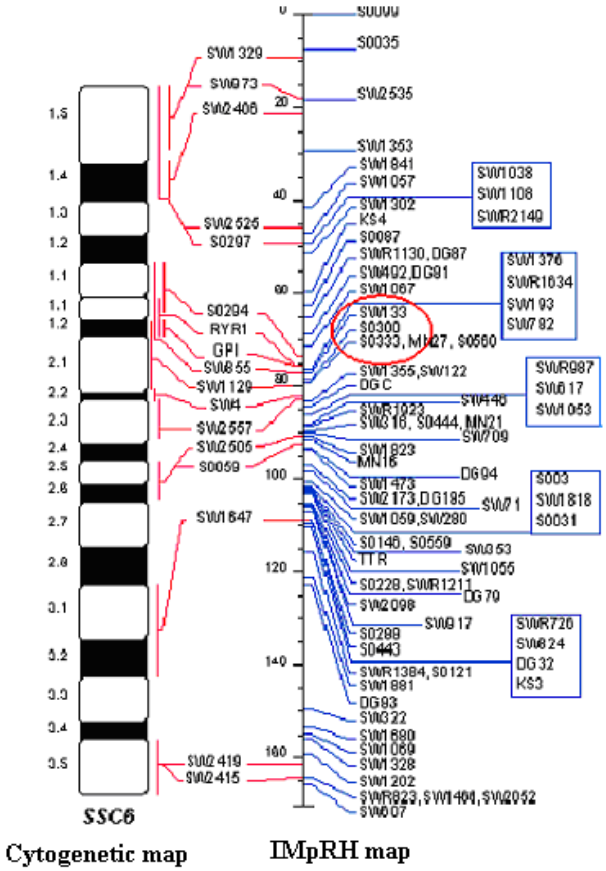

Fig 2. Localization of the Kallikrein gene family to pig Chromosome 6q12. The kallirein region is circled in red. The Radiation Hybrid map is compared with the SSC6 Cytogenetic map.

Literature Cited

Bhoola, K. D. et al. 1992. Pharmacological Reviews 44:1-80.

Emanueli, C. et al. 2001. Trends in Pharmacological Sciences 22:478-484.

Fabre, J. E. et al. 1999. Circulation 99:3043-3049.

Fronicke, L. et al. 1996. Mammalian Genome 7: 285-290.

Gan, L. et al. 2000. Gene 257:119-130.

Goureau, A. et al. 1996. Genomics 36:252-262.

Hawken, R. J. et al. 1999. Mammalian Genome 10:824-830.

Marra, M. A. et al. 1997. Genome Res. 7:1072-1084.

Sambrook, J. and D. W. Russel. 2001. Molecular cloning: a laboratory manual.

Southardsmith, M. et al. 1994. Genomics 22:404-417.

Yousef, G. M. et al. 2003. Clinical Biochemistry 36:443-452.

Authors

Fernando, S.C. – Graduate Student

Geisert, R.D. - Professor

DeSilva, U. – Assistant Professor