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FISH-BASED COMPARATIVE ANALYSIS OF HUMAN AND PORCINE CHROMOSOME REGION INVOLVING OBESITY-RELATED GENES*

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Abstract

The aim of this study was to analyse homology between the subtelomeric region of human chromosome 3 (HSA3p25-26) involving loci of obesity-related genes and a corresponding fragment in the porcine genome using FISH technique. The human subregional chromosome painting probe (HSA3p25-26) was used for hybridization with pig chromosomes. The results obtained showed strong fluorescence signals in human chromosome subtelomeric region 3p25-26 and in pig autosomal interstitial region 13q31-32. Some aspects of the homology between the human and pig chromosome segments have been discussed.

Key words: comparative FISH mapping, cross-species chromosome painting, ghrelin gene, human, pig

Advanced comparative mapping of the human and pig genome in the recent past made it possible to investigate pig genome structure, function and evolution, understand the genetic variation underlying economically important traits for pig improvement, and develop pig models for unravelling the genetic complexity of human diseases such as obesity, diabetes, cancer, cardiovascular and infectious disease, and female reproductive disorders (Chen et al., 2007; Jiang and Rothschild, 2007; Lunney, 2007).

One of the techniques developed in order to precisely identify segmental chromosome homology between humans and pigs was cross-species chromosome painting according to ZOO-FISH protocol (Scherthan et al., 1994; Rettenberger et al., 1995; Frönicke et al., 1996). The significance of this approach has been emphasized mainly for searching genomic regions and candidate *loci* governing traits of biological or economic importance, with increasing interest in mapping of quantitative trait *loci* (QTL) for growth and obesity in these species (Kim et al., 2004; Rothschild et al., 2007).

The aim of this study was to analyse homology between the subtelomeric region of human chromosome 3 (HSA3p25-26) involving *loci* of obesity-related genes and a corresponding fragment in the porcine genome using the FISH technique.

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Material and methods

Metaphase chromosome spreads of the human and domestic pig were obtained from (pokeweed mitogen stimulated) peripheral blood lymphocyte culture according to the routine protocol. The 3p SpectrumGreen TelVysion Probe, locus: 3PTEL25 (D3S4559), probe size: 80 kb (Vysis.Abbott, USA) was used (Cat.#: 33-252003, Genos, Poland). The probe did not require further treatment and it was ready to use (directly onto slide). Fluorescence *in situ* hybridization was performed according to the manufacturer's procedure. DAPI-banding was applied to precisely identify the chromosome subregion. Hybridization signals were observed under an OPTON-Axiophot fluorescent microscope using double attenuation filters – DAPI/FITC (Carl Zeiss Filterset 40: excitation – 496 nm; emission – 560 nm). Selected cells were recorded and evaluated using the image analysis software LUCIA – FISH (Laboratory Imaging Ltd., Prague, Czech Republic).

Results

The results presented in Figure 1 show distinct fluorescence signals in porcine chromosome q-arm interstitial region 13q31-32 (SSC13q31-32) (A) and corresponding human chromosome p-arm subtelomeric region 3p25-26 (HSA3p25-26) (B), pointed out also on HSA3 and SSC13 ideograms (C), according to the "Human – pig compared maps based on SSC13" (https://www-lgc.toulouse.inra.fr/pig/compare/SSCHTML/SSC13S.HTM).

Discussion

Significant progress in human/pig comparative mapping has been made by bidirectional heterologous chromosome painting, followed by somatic cell or radiation hybrid panels application, and supplemented by linkage analysis (Goureau et al., 1996; Frönicke et al., 1996; Yerle et al., 1996, 2002). Construction of these well-integrated cytogenetic and genetic maps contributed to the assignment of 170 conserved chromosomal blocks or syntenic segments in genomes of these species (Milan et al., 2006; Chen et al., 2007; Jiang and Rothschild, 2007; Rothschild et al., 2007).

Both molecular cytogenetic techniques and comparative genetic linkage maps defined complete synteny conservation between human chromosome 3 (HSA3) and pig chromosome 13 (SSC13) (encompassing seven conserved segments) but revealed several intrachromosomal rearrangements and extensive gene-order differences within this large synteny group (Johansson et al., 1995; Milan et al., 1996; Chowdhary et al., 1998; Sun et al., 1999; Van Poucke et al., 2001; 2003; Jiang and Rothschild, 2007). However, it is anticipated that the application of higher-resolution subchromosomal FISH (by using heterologous DNA probes specific for chromosome subregions with resolution of up to 10–15 Mbp) will lead to the identification of more refined segmental homology and the making of a HSA3/SSC13 comparative map suitable for QTL studies (Müller et al., 2000; Rothschild et al., 2007).

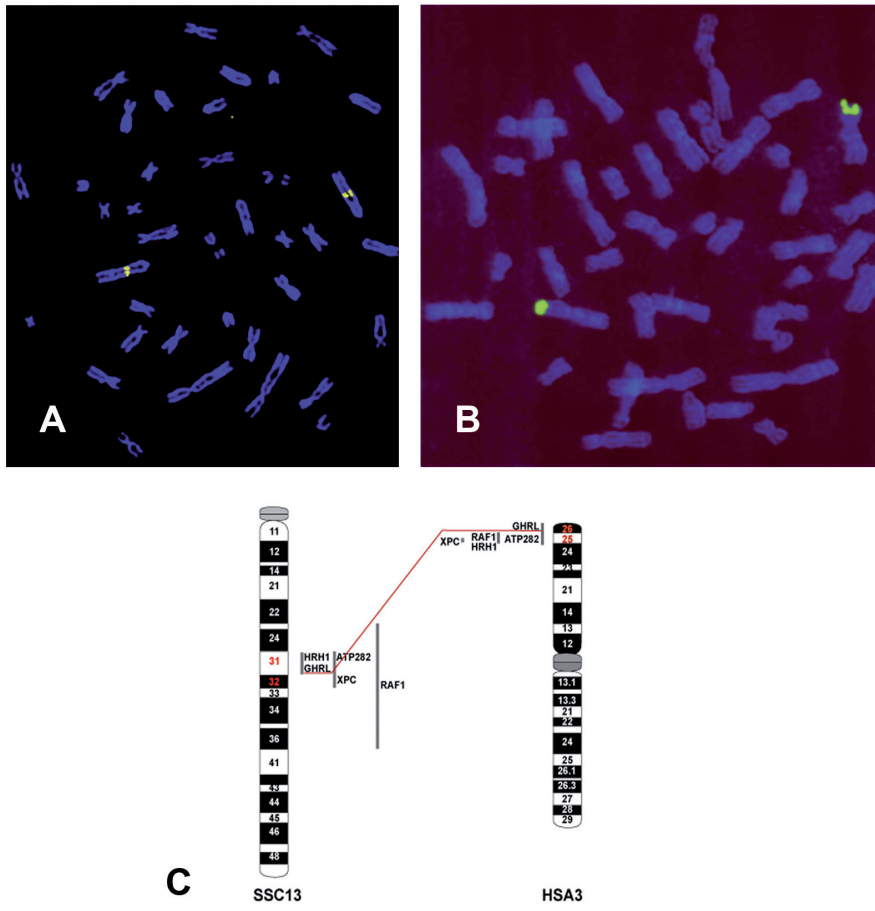

Figure 1. Fluorescence signals in porcine chromosome q-arm interstitial region 13q31-32 (SSC13q31-32) (A), and corresponding human chromosome p-arm subtelomeric region 3p25-26 (HSA3p25-26) (B), shown in red on HSA3 and SSC13 ideograms (C) (according to the "Human – pig compared maps based on SSC13", https://www-lgc.toulouse.inra.fr/pig/compare/SSCHTML/SSC13S.HTM)

The cross-species FISH mapping with human chromosome subregional probe (80 kb) presented in this paper proved total correspondence and homology between human and pig chromosomal segments HSA3p26-25 and SSC13q31-32, containing several functionally important genes: *ATP2B2* – ATPase, Ca++ transporting, plasma membrane 2; *HRH1* – Histamine H1 Receptor; *RAF1* – v-Raf-1 murine leukemia viral oncogene; *XPC* – Xeroderma pigmentosum group C and *GHRL* – Ghrelin / obestatin prepropeptide (according to comparative gene map database: https://www-lgc.toulouse.inra.fr/pig/compare/HSA.htm, http://www.ncbi.nlm.nih.gov/projects/genome/guide/pig/).

Based on the contemporary literature, it was shown that the ghrelin gene (*GHRL*) contributed a series of biological functions including regulation of food intake, body weight, gastrointestinal (GI) motility, enzyme and hormone secretion, glucose release, cardiovascular functions, cell proliferation (adipocytes, hepatocytes) and reproduction in pigs (Dong et al., 2009). For this reason, *GHRL* gene enclosed in the SSC13q31-32 region has been taken into account as a putative major gene for QTL affecting fatty acid composition, combining an increase of intramuscular fat content (IMF) enhancing monounsaturated fatty acid percentage in different pig breeds (Sanchez et al., 2007). Location of porcine *GHRL* gene confirmed by cross-species chromosome painting described in our paper will be helpful in more accurate mapping of this QTL, which would be of great interest in the pig because IMF is defined to play a key role in organoleptic meat quality. The increase of IMF is associated with an improvement in consumer perception of texture and taste. Thus, in Large White and Landrace breeds, increasing IMF content (at least in the *Longissimus dorsi* muscle) is reported as highly desirable. Additionally, not only the amount of IMF has to be considered but also fatty acid composition, which is known to affect human health and also technological quality of fresh meat and sensory value of pig meat products (Sanchez et al., 2007).

On the other hand, extensive studies have recently been focused on candidate genes known to be related with human obesity that were located in previously identified QTL regions for obesity-related traits in the pig (Kim et al., 2007). The comparative location of candidate genes associated with pig QTL regions for body composition, growth, and muscle traits can be used as anchor *loci* to find homologous human chromosomal locations and additional candidate genes. Of special interest is pig chromosome 13, especially SSC13q31-32 region with *GHRL locus*, which was shown to be completely homologous to HSA3p26-25 in our interspecies comparative FISH studies. The results obtained can help to identify genes responsible for the human obesity-related QTL on chromosome 3. Comparative analysis of obesity-related genes in pigs is not only important for development of marker-assisted selection on growth and fat deposition traits in the pig but also provides for an understanding of their genetic roles in the development of human obesity (Kim et al., 2007).

The studies reported in this paper demonstrate the usefulness of comparative human/pig analysis based on modern techniques (sequence analysis as well as FISH) to further integrate genomics of these species.

References

Chen K., Baxter T., Muir W.M., Groenen M.A., Schoock L.B. (2007). Genetic resources, genome mapping and evolutionary genomics of the pig (*Sus scrofa*). Int. J. Biol. Sci., 3: 153–165.

Chowdhary B.P., Raudsepp T., Frönicke L., Scherthan H. (1998). Emerging patterns of comparative genome organization in some mammalian species as revealed by Zoo-FISH. Genome Res., 8: 577–589.

Dong X.Y., Xu J., Tang S.Q., Li H.Y., Jiang Q.Y., Zou X.T. (2009). Ghrelin and its biological effects on pigs. Peptides, 30: 1203–1211.

Frönicke L., Chowdhary B.P., Scherthan H., Gustavsson I. (1996). A comparative

map of the porcine and human genomes demonstrates ZOO-FISH and gene mapping-based chromosomal homologies. Mamm. Genome, 7: 285–290.

Goureau A., Yerle M., Schmitz A., Riquet J., Milan D., Pinton P., Frelat G., Gellin J. (1996). Human and porcine correspondence of chromosome segments using bidirectional chromosome painting. Genomics, 36: 252–262.

Jiang Z., Rothschild M.F. (2007). Swine genome science comes to age. Int. J. Biol. Sci., 3: 129–131.

Johansson M., Ellegren H., Andersson L. (1995). Comparative mapping reveals extensive linkage conservation but with gene order rearrangements between the pig and the human genomes. Genomics, 25: 682–690.

Kim K.S., Thomsen H., Bastiaansen J., Nguyet N.T., Dekkers J.C.M., Plastow G.S., Rothschild M.F. (2004). Investigation of obesity candidate genes on porcine fat deposition quantitative trait *loci* regions. Obes. Res., 12: 1981–1994.

Lunney J.K. (2007). Advances in swine biomedical model genomics. Int. J. Biol. Sci., 3: 179–184.

Milan D., Beever J., Lahbib Y., Schook L., Beattie C., Yerle M. (2006). An integrated RH map of the porcine genome with more than 5000 anchoring points on the human genome provides a framework for sequencing of the pig. Proceedings of the 30th International Conference on Animal Genetics, 20–25.08.2006, Porto Seguro, Brazil.

Milan D., Riquet J., Yerle M., Goureau A., Schmitz A., Cribiu E.P., Frelat G., Gellin J. (1996). Homologous and heterologous FISH painting with PARM-PCR chromosome-specific probes in mammals. Mamm. Genome, 7: 194–199.

Müller S., Stanyon R., Finelli P., Archidiacono N., Wienberg J. (2000). Molecular cytogenetic dissection of human chromosomes 3 and 21 evolution. Proc. Nat. Acad. Sci., 97: 206–211.

Rettenberger G., Klett C., Zechner U., Kunz J., Vogl W., Hameister H. (1995). Visualization of the conservation of synteny between humans and pigs by heterologous chromosomal painting. Genomics, 26: 372–378.

Rothschild M.F., Hu Z.L., Jiang Z. (2007). Advances in QTL mapping in pigs. Int. J. Biol. Sci., 3: 192–197.

Sanchez M.P., Iannuccelli N., Basso B., Bidanel J.P., Billon I., Gandemer G., Gilbert H., Larzul C., Legault C., Riquet J., Milan D., Le Roy P. (2007). Identification of QTL with effects on intramuscular fat content and fatty acid composition in a Duroc × Large White cross. BMC Genetics, 8: 55–63.

Scherthan H., Cremer T., Arnason U., Weler H.U., Lima-de-Faria A., Frönicke L. (1994). Comparative chromosome painting discloses homologous segments in distantly related mammals. Nature Genet., 6: 342–347.

Sun H.F., Ernst C.W., Yerle M., Pinton P., Rothschild M.F., Chardon P., Rogel--Gaillard C., Tuggle C.K. (1999). Human chromosome 3 and pig chromosome 13 show complete synteny conservation but extensive gene-order differences. Cytogenet. Cell Genet., 85: 273–278.

Van Poucke M., Yerle M., Tuggle C., Piumi F., Genêt C., Van Zeveren A., Peelman L.J. (2001). Integration of porcine chromosome 13 maps. Cytogenet. Cell Genet., 93: 297–303.

Van Poucke M., Yerle M., Chardon P., Jacobs K., Genêt C., Mattheeuws M., Van Zeveren A., Peelman L.J. (2003). A refined comparative map between porcine chromosome 13 and human chromosome 3. Cytogenet. Genome Res., 102: 133–138.

Yerle M., Echard G., Robic A., Mairal A., Dubut-Fontana C., Riquet J., Pinton P., Milan D., Lahbib-Mansais Y., Gellin J. (1996). A somatic cell hybrid panel for pig regional gene mapping characterized by molecular cytogenetics. Cytogenet. Cell Genet., 73: 194–202.

Yerle M., Pinton P., Delcros C., Arnal N., Milan D., Robic A. (2002). Generation and characterization of a 12,000-rad radiation hybrid panel for fine mapping in pig. Cytogenet. Genome Res., 97: 219–228.

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Analiza porównawcza regionu chromosomowego zawierającego geny warunkujące otyłość człowieka i świni z wykorzystaniem techniki FISH

STRESZCZENIE

Celem badań było wykorzystanie techniki FISH do analizy homologii między subtelomerowym regionem chromosomu 3 człowieka (HSA3p25-26), w którym zlokalizowane są geny warunkujące otyłość, a odpowiadającym mu fragmentem genomu świni. Do hybrydyzacji z chromosomami świni wykorzystano ludzką sondę malującą specyficzną dla subregionu autosomu 3 (HSA3p25-26). Uzyskano wyraźne sygnały fluorescencyjne w subtelomerowym regionie ludzkiego chromosomu 3p25-26, a także w interstycjalnym regionie autosomalnym 13q31-32 u świni. Omówiono zjawisko homologii pomiędzy segmentami chromosomów człowieka i świni.