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A novel gene on human chromosome 2p24 is differentially expressed between androgen-dependent and androgen-independent prostate cancer cells

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Abstract

Identification of genes involved in the transition from androgen-dependent to androgen-independent prostate cancer is important to extend our current knowledge of the disease. Using differential display RT-PCR analysis between androgen-dependent and androgen-independent prostate cancer cells, we have identified a novel gene, designated *GC109*. *GC109* harbours a putative Cys-His cluster, a nuclear localisation signal, a leucine zipper and a ret finger protein (rfp)-like domain. *GC109* mRNA expression in normal human tissues was found not to be restricted to the prostate. However, using a variety of 15 human cancer cell lines, *GC109* mRNA was preferentially expressed in androgen-dependent LNCaP-FGC, compared with androgen-independent LNCaP-LNO, DU145 and PC3 human prostate cancer cells. Finally, the *GC109* gene was mapped on human chromosome 2p24. Based on its protein domain structure and chromosomal localisation, we hypothesise that *GC109* may be involved in chromosomal rearrangements in prostate cancer.

Keywords: Differential gene expression; Androgen-independent; Prostate cancer; Chromosome 2p24

1. Introduction

Prostate cancer is the second leading cause of male cancer-related deaths and the most commonly diagnosed cancer in elderly men in the Western world [1]. Prostate cancer is initially an androgen-dependent disease and can be treated effectively by inhibiting endogenous androgen production or action. However, it has been reported that in an animal model, almost all prostate tumours will progress, which results in growth of androgen-independent cells and subsequent development of hormone-refractory prostate disease. The mechanism of prostate cancer progression from an androgen-dependent to an androgen-independent state is not fully understood [2]. Genetic alterations (loss and gain) most likely play a role during prostate cancer progression. It has been reported that major genetic alterations occur during androgen-dependent prostate cancer growth, and minor genetic alterations occur during androgen-independent prostate cancer growth [3,4]. Identification of genetic factors that are differentially expressed between androgen-dependent and androgen-independent prostate cancer may be important to extend our current knowledge of the molecular mechanism(s) underlying the development of hormone-refractory prostate cancer.

Applying differential display reverse transcriptase-polymerase chain reaction (RT-PCR) analysis to androgen-dependent and androgen-independent prostate cancer cells, we have identified and cloned several cDNA transcripts [5–7]. Here, we report on the identification of a novel gene, designated *GC109*. *GC109* is localised on the human chromosome 2p24 locus and was found to be expressed more highly in androgen-

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dependent compared with androgen-independent prostate cancer cell lines.

2. Materials and methods

2.1. Human cancer cell lines

The lymph node carcinoma of the prostate-fast growing colony (LNCaP-FGC) and lymph node carcinoma of the prostate-lymph node original (LNCaP-LNO) cell lines [8] were gifts from Dr J.S. Horoszewicz (Buffalo, NY, USA) and were propagated as described in Ref. [5,9].

The human cancer cell lines PC3 and DU145 (androgen-independent prostate carcinomas), ZR-75-1 (oestrogen-dependent breast carcinoma), T47D and MCF7 (oestrogen-responsive breast carcinomas), MDA-MB-231 and SKBR-3 (oestrogen-independent breast adenocarcinomas), HeLa (cervical carcinoma), IMR32 (neuroblastoma), HEPG2 (hepatoma), T24 (bladder carcinoma), SnuC1 and SnuC5 (colon carcinomas) were cultured in their appropriate medium as recommended by the American Type Culture Collection (ATCC, Rockville, MD, USA).

2.2. RNA arbitrary primed and differential display RT-PCR analysis

Total RNA from 6 days LNCaP-FGC and LNCaP-LNO cell cultures was isolated for differential display RT-PCR analysis as described in Ref. [5] using a single arbitrary primer (HB25, 5′-GCAAGCTTGCGATCA-TAGCG-3′, Pharmacia Biotech, Roosendaal, The Netherlands). The differentially expressed *GC109* fragment was 390 bp long, ^{32}P-labelled and used as probe to screen an oligo(dT) + random primed human prostate 5′-STRETCH cDNA library λgt10 (Clontech, Palo Alto, CA, USA). The screen yielded 4 positive clones and the largest clone (pCR2 109 5R1) had an insert of 957 nucleotides.

2.3. Northern and dot blot analyses

Northern blot analysis was performed as described in Ref. [5]. A human dot blot was obtained (Master Blot, Clontech, Palo Alto, CA, USA) and contained poly A^+ RNA (range: 89–514 ng/dot) isolated from 50 different human normal tissues. The amounts of RNA on the blot were normalised against eight different housekeeping genes and the source of RNA was obtained from individuals who died of trauma. Hybridisation using ^{32}P-labelled 390 bp *GC109* bp probe was performed according to the manufacturer's instructions. The blot was exposed to an X-ray film (Kodak X-Omat, Rochester, NY, USA) at −80 °C with intensifying screens for at least 18 h.

2.4. Chromosomal localisation

The 390 bp *GC109* fragment was ^{32}P-labelled and used to screen a genomic human PAC library on gridded filters (Genome Systems, St. Louis, MO, USA). Hybridisation was performed according to the manufacturer's instructions. From positive PAC clones, genomic DNA was isolated according to the protocol of the manufacturer and used for fluorescence *in situ* hybridisation (FISH) analysis as described in Refs. [6,7]. Analysis was performed using a Leica DM-RXA microscope equipped with a PowerGene Image analysis system (PSI, Chester, UK).

3. Results

3.1. GC109 is differentially expressed between LNCaP-FGC and LNCaP-LNO cells

Two genetically related LNCaP cell lines were used to isolate genes involved in androgen-independent prostate cancer development. The LNCaP-FGC cell line is androgen-dependent and has an optimal growth rate at 10^{-10} M R1881 (synthetic androgen), whereas higher concentrations did not stimulate growth at all. Both 10^{-7} M 1.25 $(OH)_2D_3$ (vitamin D_3) or a combination of 10^{-8} M R1881 plus 10^{-7} M 1.25 $(OH)_2D_3$ did not stimulate growth (data not shown). The LNCaP-LNO cell line is androgen-independent and growth is slightly inhibited with concentrations higher than 10^{-8} M R1881. A combination of 10^{-8} M R1881 with 10^{-7} M 1.25 $(OH)_2D_3$ inhibits the growth of LNCaP-LNO cells almost completely (data not shown). 1.25 $(OH)_2D_3$ served as a tool to obtain conditions under which both cell lines were growth-arrested [5]. Differential display RT-PCR analysis was performed using controlled cell culture conditions with respect to growth: 0, 10^{-10} M R1881, 10^{-8} M R1881, 10^{-7} M 1.25 $(OH)_2D_3$ and a combination of 10^{-7} M 1.25 $(OH)_2D_3$ with 10^{-8} M R1881. The relevant differentially expressed fragments were isolated, amplified, cloned and expression was confirmed by Northern blot analysis (Fig. 1). Relative to the amount of total RNA, *GC109* mRNA is more highly expressed in the LNCaP-FGC cells (lanes 1–5) compared with the LNCaP-LNO cells (lanes 6–10). *GC109* mRNA seems not to be regulated by either R1881 (lanes 2 and 3) or 1.25 $(OH)_2D_3$ (lane 4), or a combination of R1881 plus 1.25 $(OH)_2D_3$ (lane 5).

3.2. Expression of GC109 mRNA in human normal tissues

The tissue specificity of *GC109* mRNA expression was examined using a human tissue dot blot containing poly A^+ RNA isolated from normal individuals. The

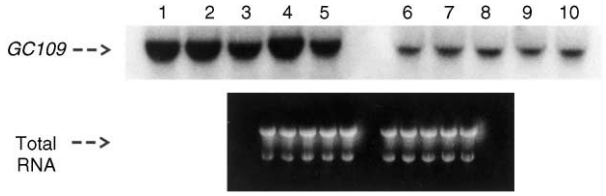

Fig. 1. Northern blot analysis of *GC109* mRNA expression in human LNCaP-FGC and LNCaP-LNO prostate cancer cells. Total RNA (20 μg) was loaded on each lane of a 1.5% denatured agarose gel, electrophoresed, blotted onto nylon filters and hybridised with the ^{32}P-labelled 390 bp *GC109* probe. Each lane contains an equal amount of total RNA as measured by absorbance at 260 nm and ethidium bromide staining. Lanes 1–5, androgen-dependent LNCaP-FGC cells; lanes 6–10, androgen-independent LNCaP-LNO cells; lanes 1 and 6, no additions; lanes 2 and 7, 10^{-10} M R1881; lanes 3 and 8, 10^{-8} M R1881; lanes 4 and 9, 10^{-7} M 1.25 $(OH)_2D_3$; lanes 5 and 10, 10^{-8} M R1881 plus 10^{-7} M 1.25 $(OH)_2D_3$.

amount ranged from 89 to 514 ng/dot, which was normalised against eight different housekeeping genes. Fig. 2 shows, that besides expression in the prostate (C7), *GC109* mRNA expression was observed at comparable levels in heart (C1), colon (C4), pituitary gland (D4), adrenal gland (D5), thyroid gland (D6), kidney (E1), liver (E2), small intestine (E3), spleen (E4), thymus (E5), trachea (F3), placenta (F4) and foetal kidney (G3).

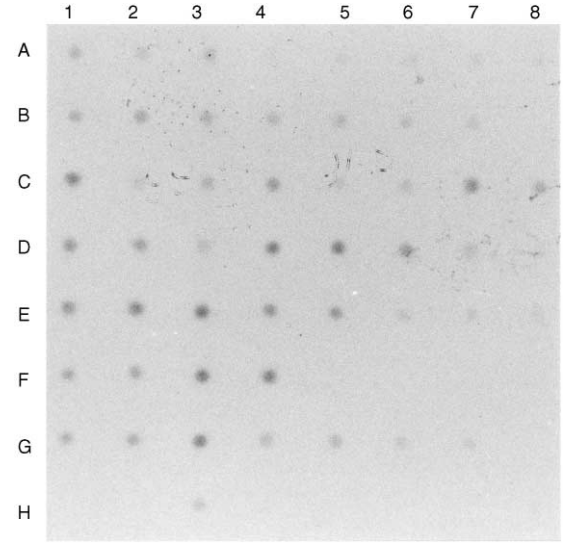

Fig. 2. Dot blot analysis of *GC109* mRNA expression in human normal tissues. A dot blot containing poly A^+ RNA (range: 89–514 ng/dot) from 50 different normal human tissues was hybridised with the ^{32}P-labelled 390 bp probe *GC109*. Row A: 1, whole brain; 2, amygdala; 3, caudate nucleus; 4, cerebellum; 5, cerebral cortex; 6, frontal lobe; 7, hippocampus; 8, medulla oblongata. Row B: 1, occipital lobe; 2, putamen; 3, substantia nigra; 4, temporal lobe; 5, thalamus; 6, subthalamic nucleus; 7, spinal cord. Row C: 1, heart; 2, aorta; 3, skeletal muscle; 4, colon; 5, bladder; 6, uterus; 7, prostate; 8, stomach. Row D: 1, testis; 2, ovary; 3, pancreas; 4, pituitary gland; 5, adrenal gland; 6, thyroid gland; 7, salivary gland; 8, mammary gland. Row E: 1, kidney; 2, liver; 3, small intestine; 4, spleen; 5, thymus; 6, peripheral leucocyte; 7, lymph node; 8, bone marrow. Row F: 1, appendix; 2, lung; 3, trachea; 4, placenta. Row G: 1, fetal brain; 2, fetal heart; 3, fetal kidney; 4, fetal liver; 5, fetal spleen; 6, fetal thymus; 7, fetal lung. Row H: 1, yeast total RNA, 100 ng; 2, yeast tRNA, 100 ng; 3, *Escherichia coli* rRNA, 100 ng; 4, *E. coli* DNA, 100 ng; 5, Poly r(A), 100 ng; 6, human C_ot 1 DNA, 100 ng; 7, human genomic DNA, 100 ng; 8, human genomic DNA, 500 ng.

3.3. *Expression of* GC109 *mRNA in established human cancer cell lines of different origin*

GC109 mRNA expression was examined in 15 established human cancer cell lines of different origin. Fig. 3a shows that the highest expression was present in human androgen-dependent LNCaP-FGC cells (lane 1) and found to be reduced in androgen-independent prostate cancer cell lines LNCaP-LNO, PC3 and DU145 (lanes 2–4, respectively). Furthermore, *GC109* mRNA expression was reduced or absent in the human HeLa, SnuC1, SnuC5, IMR32, HEPG2 and T24 cancer cell lines (lanes 5–10, respectively). Finally, Fig. 3b shows that *GC109* mRNA expression was reduced or absent in the human breast cancer cell lines ZR-75-1, T47D, MCF7, MDA-MB-231 and SKBR3 (lanes 11–15, respectively). Together, these results show that overexpression of *GC109*

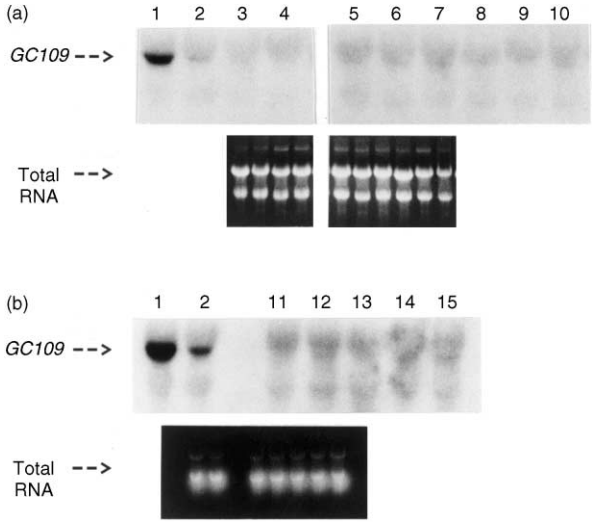

Fig. 3. Northern blot analysis of *GC109* mRNA expression in established human cancer cell lines. Total RNA (20 μg) was loaded on each lane of a 1.5% denatured agarose gel, electrophoresed, blotted onto nylon filters and hybridised with the ^{32}P-labelled 390 bp *GC109* probe. Each lane contains an equal amount of total RNA as measured by absorbance at 260 nm and ethidium bromide staining. Lane 1, LNCaP-FGC (androgen-dependent prostate carcinoma); lane 2, LNCaP-LNO (androgen-independent prostate carcinoma); lane 3, PC3 (androgen-independent prostate carcinoma); lane 4, DU145 (androgen-independent prostate carcinoma); lane 5, HeLa (cervical carcinoma); lane 6, SnuC1 (colon carcinoma); lane 7, SnuC5 (colon carcinoma); lane 8, IMR32 (neuroblastoma); lane 9, HEPG2 (hepatoma); lane 10, T24 (bladder carcinoma); lane 11, ZR-75-1 (oestrogen-dependent breast carcinoma); lane 12, T47D (oestrogen-responsive breast carcinoma); lane 13, MCF7 (oestrogen-responsive breast adenocarcinoma); lane 14, MDA-MB-231 (oestrogen-independent breast adenocarcinoma) and lane 15, SKBR-3 (oestrogen-independent adenocarcinoma).

may be restricted to androgen-dependent LNCaP-FGC prostate cancer cells.

3.4. Cloning and sequencing of GC109

The differentially expressed cDNA clone *GC109* was sequenced in both directions and was 390 bp long. Screening of a human prostate cDNA λgt10 library yielded a clone (pCR2 109 5R1) of 957 bp. Fig. 4 shows the nucleotide sequence of clone pCR2 109 5R1. A homology search in the EMBL/GenBank databases was performed, using the BLAST Server [10]. Translation of the nucleotide sequence reveals a novel gene with an open reading frame of 319 amino acid residues. Domain analysis reveals homology to a family of proteins containing a RING finger, B1 and B2 boxes, a coiled coil and a rfp-like or B30.2 domain [11–13]. The N-terminal part of *GC109* has a Cys-His cluster (Cys-X2-Cys-X5-His-X2-His) that may represent a part of the B2 box domain [12], a bipartite nuclear localisation signal domain [14], and a coiled coil domain that represents a leucine zipper domain [15,16]. The C-terminal part of *GC109* has high homology with a domain, that is originally found in human *ret* finger protein (RFP) [17]. The protein members of this family will be tentatively referred to as the RING Bbox Coil Ret (RBCR) family.

3.5. Chromosomal localisation of GC109

Southern blot analysis using the ^{32}P-labelled 390 bp *GC109* probe on LNCaP-FGC genomic DNA digested with either *Hind*III, or *Eco*RI or *Bam*HI, demonstrated that a single copy gene encodes *GC109* (data not shown). Screening of a human genomic PAC library yielded six positive clones (2L8, 14F17, 53N3, 133H7, 195O3 and 224B24). Genomic DNA was isolated from PAC clone 2L8, verified by PCR and used as a probe on metaphase spread chromosomes using fluorescence *in situ* hybridisation analysis. The detected signals were localised on the human chromosome 2p24 locus (Fig. 5).

4. Discussion

Using differential display RT-PCR analysis and a controlled cell culture system, *GC109* was identified (Fig. 1). *GC109* mRNA is higher expressed in androgen-dependent LNCaP-FGC compared with androgen-independent LNCaP-LNO prostate cancer cells. In normal human tissues, *GC109* mRNA expression was not limited to the prostate, but was also observed in various other human tissues (Fig. 2). In fetal tissues, expression was in general lower compared with their normal human counterparts, except for fetal kidney (G3). Next, we examined *GC109* mRNA expression in several established human cancer cell lines of various origins (Fig. 3). *GC109* was more highly expressed in androgen-dependent LNCaP-FGC, compared with androgen-independent LNCaP-LNO, PC3 and DU145 prostate cancer cell lines. This demonstrates that expression of *GC109* is decreased, and that *GC109* might be involved during prostate cancer progression. Because, in other cancer cell lines, *GC109* was hardly present, these observations suggest that overexpression of *GC109* may be restricted to androgen-dependent LNCaP-FGC prostate cancer cells. In addition, since *GC109* mRNA expression does not seem to be affected by androgens (Fig. 1), *GC109* may be a novel tumour marker to study prostate cancer progression in patients with different stages of disease. The comparison of the expression of the gene in clinical samples of hormone-dependent and hormone-independent prostate cancers would help to provide supporting data for this hypothesis.

Partial cloning showed that *GC109* was a novel gene, belonging to the RBCR family members of proteins with oncogenic potential. The N-terminal part of the RBCR family, that are able to fuse to other proteins, include the the *ret* finger protein (RFP), promyelocytic leukaemia protein (PML) and transcriptional intermediary factor 1 (TIF1) among others [11–13,17]. PML is associated with acute promyelocytic leukaemia (APL) and can fuse with retinoic acid receptor α (RARα), involved in the chromosomal translocation t(15;17)(q22q21). TIF1 can interact with several nuclear receptors *in vivo* and seems to mediate the ligand-dependent transcriptional activity of nuclear receptors. The N-terminal part of TIF1 can fuse with the serine/threonine kinase domain (B-raf) of the mouse oncogenic protein T18. RFP can fuse with the tyrosine kinase domain of the proto-oncogene *ret* to form the *ret* transforming gene [17]. Recently, the ret protein has been reported to be involved in prostate cancer [18]. Furthermore, the RBCR family includes human ribonucleoprotein Ro/SSA, the autoantigen A involved in Sjögren's Syndrome and systemic lupus erythematosus (SLE); a regulatory protein of murine T-lymphocyte 1 (rpt-1), which is a downregulator of interleukin-2; stimulated *trans*-acting factor of 50 kDa (Staf-50), an interferon-inducible transcriptional regulator; acid finger protein (AFP) implicated in major histocompatibility complex (MHC) class 1 and oestrogen-responsive finger protein (EFP). Recently, mutations have been found in novel members of the RBCR family. Pyrin or marenostrin, has been reported to be involved in familial Mediterranean fever which is characterised by recurrent attacks of inflammation. Midline 1 (*MID1*) is a gene implicated in Opitz G/BBB syndrome, and is characterised by an inherited multiple-organ disorder, that affects midline development. In addition to *MID1*, a novel homologue, *MID2*, has been reported. Non-

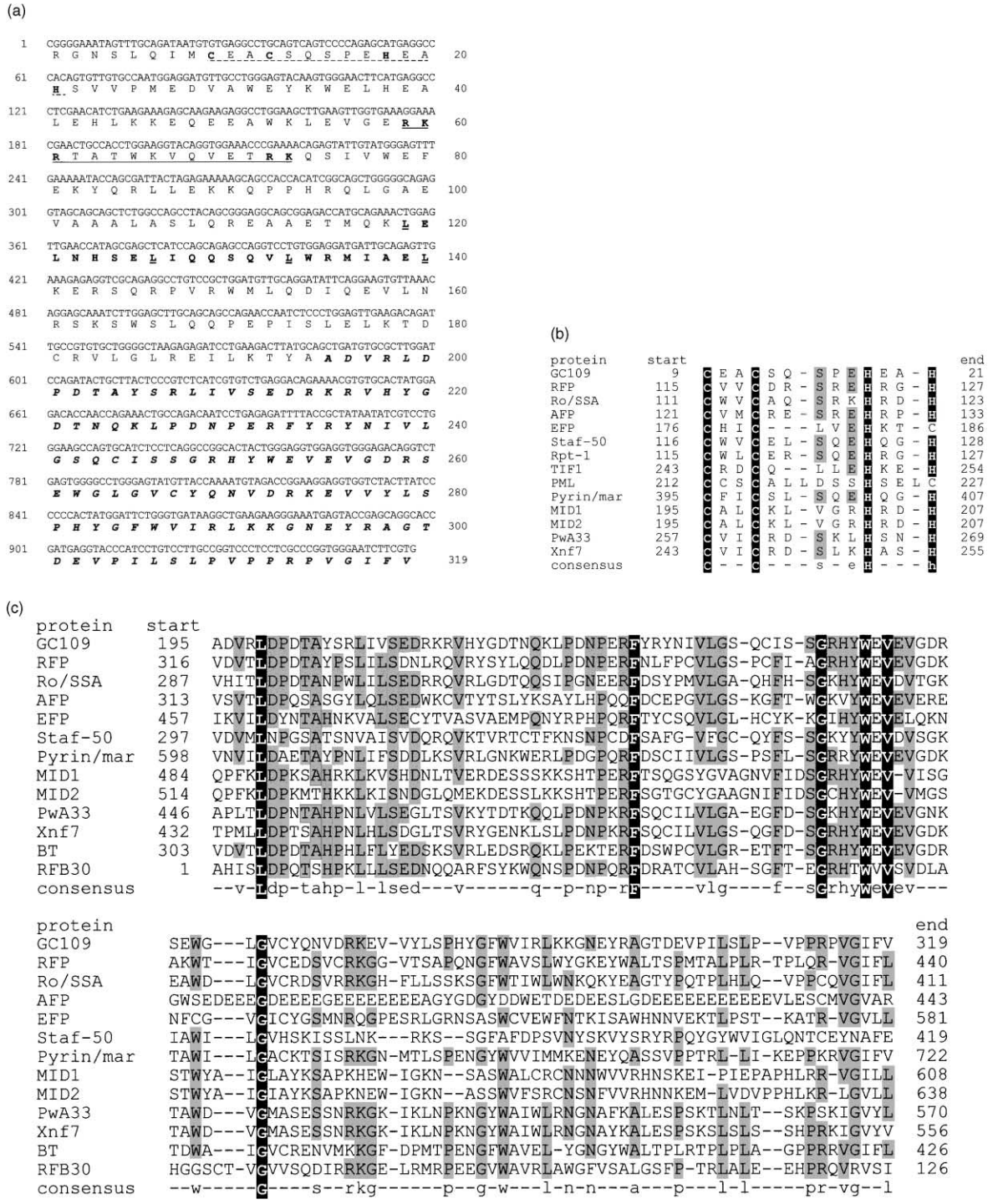

Fig. 4. Nucleotide and deduced amino acid sequences of clone *GC109*. (a) The cDNA clone pCR2 109 5R1 was isolated from a human prostate cDNA library and sequenced using the dideoxy chain-terminating method. The numbering of the nucleotide sequence is on the left margin and the amino acid sequence is on the right margin. Putative Cys-His cluster is indicated by a dotted line, nuclear localisation signal is underlined, leucine zipper domain is in bold and ret finger protein (rfp)-like domain is in bold and italics. (b) Alignment of Cys-His cluster of GC109 with other proteins. (c) Comparison of proteins harbouring a rfp-like domain. The alignments in (b) and (c) show GC109 (this report); ret finger protein (RFP) [7]; ribonucleoprotein (Ro/SSA); acid finger protein (AFP); oestrogen-responsive finger protein (EFP); stimulated *trans*-acting factor (Staf-50); promyelocytic leukaemia protein (PML); transcriptional intermediary factor 1 (TIF1); regulatory protein (rpt-1); pyrin; marenostrin; midline1 protein (MID1); midline2 protein (MID2); zinc finger protein (PwA33); nuclear protein (xnf7); butyrophilin (BT) and ret finger protein (RFB30). GenBank Accession number: AY005802.

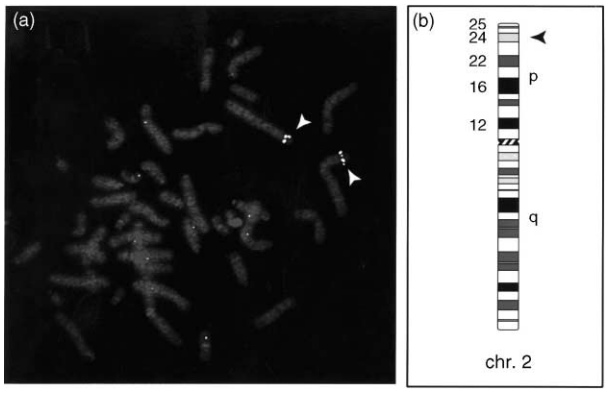

Fig. 5. Chromosomal localisation of *GC109*. (a) *In situ* hybridisation of digoxigenin-labelled *GC109* PAC clone (2L8) on human metaphase spread chromosomes. Specific hybridisation was observed on both chromosomes 2 at band p24 (double dots at white arrows). (b) A representing idiogram of chromosome 2 showing the 2p24 locus. Images were obtained using a Leica DM-RXA microscope.

mammalian members of the RBCR family include a nuclear factor (xnf7) that binds mitotic chromosomes of the frog *Xenopus Laevis* and a zinc finger protein (PwA33) that binds lampbrush chromosomes of the newt *Pleurodeles Waltl*. Interestingly, high homology, (>55%) is found with the rfp-like domain of butyrophilin (BT), a milk transmembrane glycoprotein with potential receptor function. However, BT lacks the N-terminal domain, which is a characteristic for the RBCR family members [11–13].

The *GC109* gene was mapped on human chromosome 2p24 (Fig. 5). The 2p24 locus has been reported to be frequently amplified in neuroblastoma and retinoblastoma [19,20]. Furthermore, it was reported that in the 2p24 locus, rearrangements in neuroblastomas are common [21,22]. This suggests that several genes are involved in 2p24 rearrangements.

In conclusion, our results show that *GC109* is more highly expressed in androgen-dependent compared with androgen-independent prostate cancer cells. This suggests that *GC109* may be involved in the development of androgen-independent prostate cancer. *GC109* mRNA is not affected by androgens and seems to be expressed preferentially in androgen-dependent prostate cancer cells. This demonstrates that *GC109* may be a novel tumour marker to study progression in material of prostate cancer patients with different stages of disease. *GC109* is localised on human chromosome 2p24 and is a novel member of the expanding RBCR family. The function of *GC109* in prostate and prostate cancer is currently under investigation.

Acknowledgements

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