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REGULAR ARTICLE

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Regulated expression patterns of *IRX-2*, an Iroquois-class homeobox gene, in the human breast

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Abstract In the mouse mammary gland, homeobox gene expression patterns suggest roles in development and neoplasia. In the human breast, we now identify a family of Iroquois-class (IRX) homeobox genes. One gene, *IRX-2*, is expressed in discrete epithelial cell lineages being found in ductal and lobular epithelium, but not in myoepithelium. Expression is absent from associated mesenchymal adipose stroma. During gland development, expression is concentrated in terminal end buds and terminal lobules and is reduced in a subset of epithelial cells during lactation. In contrast to observations for many homeobox genes in the mouse mammary gland in which homeobox gene expression is lost on neoplastic progression, *IRX-2* expression is maintained in human mammary neoplasias. Data suggest *IRX-2* functions in epithelial cell differentiation and demonstrate regulated expression during ductal and lobular proliferation as well as lactation.

Key words Mammary gland · Differentiation · Neoplasia · Homeodomain · Cancer · Human

Introduction

Mammary gland development in rodents and humans appears similar (Daniel and Silberstein 1987; Russo and Russo 1987) and may be divided into a proliferative and a cyclical phase of development. During the proliferative phase, ductal outgrowth begins at the nipple with subsequent invasion of the mammary adipose stroma occurring during puberty. As ductal proliferation proceeds, lumenal epithelial and myoepithelial cell differentiation occurs primarily in the growing terminal end bud located at the end of elongating ducts. In humans, terminal lobules are also formed as precursors to the mature pre-secretory lobules. The cyclical phase of development is initiated by pregnancy with terminal differentiation of lobule-alveolar secretory cells and lactation. After weaning, the cycle is completed by apoptosis (death) of secretory cells in involution. After involution, the remodeled gland resembles the morphology of the mature state.

Genetic control of mammary epithelial cell type differentiation (Smith 1996; Chepko and Smith 1997) and functional differentiation are poorly understood. Candidate regulators include homeobox genes which specify eukaryotic cell fate during development (Manak and Scott 1994; Thesleff et al. 1995). In the mouse, homeobox genes may function throughout mammary proliferative development and lactation and may contribute to development of mammary cancers (Friedmann et al. 1994; Stuart et al. 1995; Friedmann and Daniel 1996; Phippard et al. 1996). Loss-of-function of at least one homeobox gene, *Hoxd-10*, results in defects during lactation (Carpenter et al. 1997; Lewis, unpublished).

In searching nucleotide sequence databases for novel homeobox genes expressed in the human breast, we identified a partial cDNA represented as an Expressed Sequence Tag (EST) and initiated an expression study of this gene, ultimately designated *IRX-2*, in the human breast and associated cancers. In this paper, we present evidence that *IRX-2* is differentially expressed in major mammary epithelial cell lineages and that its expression is further regulated during both the proliferative and cyclical phases of human breast development.

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A

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          10         20         30         40         50         60         70         80         90
IRX1  TCCCGTCCCA AGAACGCCAC CAGGGAGAGC ACCAGCACGC TGAAGGCCTG GCTCAACGAG CACCGCAAGA ACCCCTACCC CACCAAGGGC
      *.*.**.**. .......... *.....***. ..****..*. .*........ .......... .......... .......... ..........
IRX2  GCGTACCGGA AGAACGCCAC AAGGGACGCC ACGGCTACCC TCAAGGCCTG GCTCAACGAG CACCGCAAGA ACCCCTACCC CACCAAGGGC
      .****...*. .......*.. **...**... ..***..*.. .......... .......... .......*.. .......... ...*.....*
IRX3  GGTCGCCGAA AGAACGCGAC CCGGGAGACC ACCAGTACAC TCAAGGCCTG GCTCAACGAG CACCGCAAAA ACCCCTACCC CACTAAGGGT
      ***...*..*. .......*.. *..*...... .......*.*. .......... ...**.*... .......*.. .......... ...*.....*
IRX4  ---CGGCGCA AGAACGCCAC GCGCGAGACC ACCAGCACGC TCAAGGCCTG GCTGCAGGAG CACCGCAAGA ACCCCTACCC CACCAAGGGC
      ***....*.. .......... *.......*. .......... .......... ...**.*... .......... .*........ ..........
IRX5  GGGCGGCCCA AGAACGCCAC CCGCGAGAAC ACCAGCACGC TCAAGGCCTG GCTCAACGAG CACCGCAAGA ATCCCTACCC CACCAAGGGC

         100        110        120        130        140        150        160        170        180
IRX1  GAGAAGATCA TGCTGGCCAT CATCACCAAG ATGACCCTCA CCCAGGTGTC CACC (R1 primer)
      .......... .......... .......... .......... .......... ....
IRX2  GAGAAGATCA TGCTGGCCAT CATCACCAAG ATGACCCTCA CCCAGGTGTC CACCTGGTTC GCCAACGCGC GCCGGCGCCT CAAGAAAGAG
      .......... .......... .......... .......... .......... ....
IRX3  GAGAAGATCA TGCTGGCCAT CATCACCAAG ATGACCCTCA CCCAGGTGTC CACC (R1 primer)
      .......... .......... ..*....... .......... .*.....*.. ....
IRX4  GAGAAGATCA TGCTGGCCAT CACCACCAAG ATGACCCTCA CACAGGTCTC CACC (R1 primer)
      .......... .......... ..*....... .......... .*........ ....
IRX5  GAGAAGATCA TGCTGGCCAT CATCACCAAG ATGACCCTCA CGCAGGTCTC CACC (R1 primer)
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B

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                     Hexapeptide-like                         Homeodomain
Antp        LYPWMR                          RKRGRQTYTRYQTLELEKEFHF---NRYLTRRRRIEIAHALCLTERQIKIWFQNRRMKWKKEN
mrr         YHPYD---AAFAGYPF--NSYGMDLN-GARRKNATRETTSTLKAWLNEHKKNPYPTKGEKIMLAIITKMTLTQVSTWFANARRRLKKEN
caup        YYSYDP-MSAYGGLLVSNSSYGASYDLAARRKNATRESTATLKAWLSEHKKNPYPTKGEKIMLAIITKMTLTQVSTWFANARRRLKKEN
ara         YYSYDPTLAAYG--------YGPNYDLAARRKNATRESTATLKAWLNEHKKNPYPTKGEKIMLAIITKMTLTQVSTWFANARRRLKKEN
IRX-2a      SYPY----------------GDP----AYRKNATRDATATLKAWLNEHRKNPYPTKGEKIMLAIITKMTLTQVSTWFANARRRLKKEN
IRX-3       YYPYERTLGQYQYERYGAVELSGA----GRRKNATRETTSTLKAWLNEHRKNPYPTKGEKIMLAIITKMTLTQVST (R1 primer)
IRX-4       YYPYEPALGQYPYDRYGTMD-SGT-----RRKNATRETTSTLKAWLQEHRKNPYPTKGEKIMLAIITKMTLTQVST (R1 primer)
IRX-5       YYPY----GQFQY--------GDP----GRPKNATRENTSTLKAWLNEHRKNPYPTKGEKIMLAIITKMTLTQVST (R1 primer)
IRX-1       FYPY----GQYQF--------GDP----SRPKNATRESTSTLKAWLNEHRKNPYPTKGEKIMLAIITKMTLTQVST (R1 primer)

CONSENSUS   YYPY    GQYQY        GDP     RRKNATRESTSTLKAWLNEHRKNPYPTKGEKIMLAIITKMTLTQVSTWFANARRRLKKEN
100%         +++     ++                     +++++   ++++++++ ++++++++++++++++++ ++++++++++
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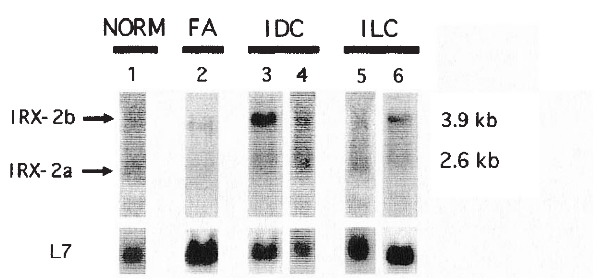
C



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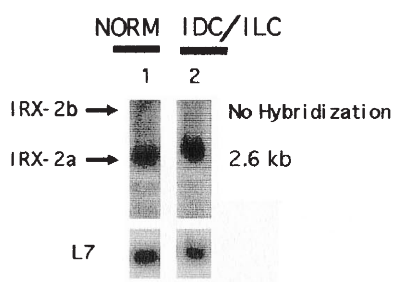

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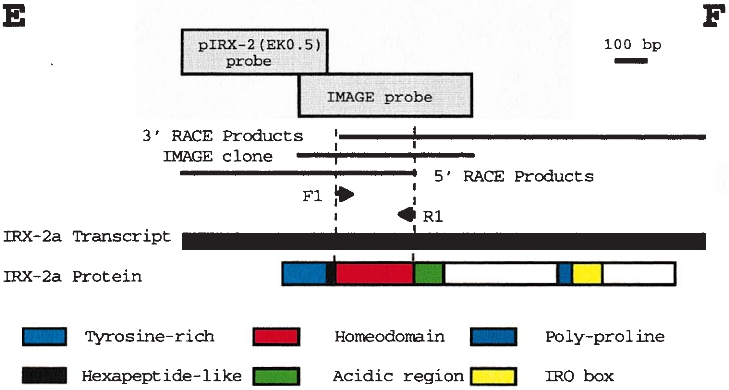

F

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MAVETTVHTHLSASPPQGSPYDHTPGMAGSLGYHPYAAPLGSYPY
GDPAYRKNATRDATATLKAWLNEHRKNPYPTKGEKIMLAIITKMT
LTQVSTWFANARRRLKKENKMTWTPRNRSEDEEEEENIDLEKNDE
DEPQKPEDKGDPEGPEAGGAEQKAASGCERLQGPPTPAGKETEGS
LSDSDFKEPPSEGRLDALQGPPRTGGPSPAGPAAARLAEDPAPHY
PAGAPAPGPHPAAGEVPPGPGGPSVIHSPPPPPPPAVLAKPKLWS
LAEIATLSDKVKDGGGGNEGSPCPPCPGPIAGQALGGSRASPAPA
PSRSPSAQCPFPGGTVLSRPLYYTAPFYPGYTNYGSFGHLHGHPG
PGPGPTTGPGSHFNGLNQTVLNRADALAKDPKMLRSQSQLDLCKD
SPYELKKGMSDI (417 a.a.)
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G

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ara      CCENGRPIMTDPVSGQT--VCSCQ
caup     RCENGRPIITDPVSGQT--VCSCQ
mrr      CCDTGRTIYTDPVSGQT--ICSCQ
IRX-3    CCESTQRSVSDVASGSTPAPALCC

CON.     CCE GR I TDPVSGQT   CSCQ
           +      +  ++ +    +
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H

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ara      KMTWEPKNRTDDDDDALVSDDEKDKEDLE
caup     KMTWEPKNKTEDDDDGMMSDDEKEKDAAD
mrr      KMTWEPRNRVDDDDANIDDDDDKNTEDND
IRX-2a   KMTWTPRNRSEDEEEEENIDLEKDNEDEP

CON.     KMTWEP NR  DDD      DDEK  ED
         ++++ + +    +       +  +
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I

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ara      KPKIWSLADTV
caup     KPKIWSVADTA
mrr      KPRIWSLADMA
IRX2a    KPKLWSLAEIA

CON.     KPKIWSLAD A
         ++  ++ +
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Materials and methods

Human tissues

Breast tissue was obtained from mastectomy or reduction mammaplasty patients in accordance with protocols and ethical standards reviewed by our Institutional Review Board. All participating patients were informed of the nature of the study and gave written consent for use of their tissues prior to surgery. For each patient and tissue type (i.e., normal or tumor), independent tissue samples were immediately frozen in liquid nitrogen (for RNA extraction) or fixed in 4% paraformaldehyde:phosphate-buffered saline (PBS) (for in situ hybridization). Tumors were either non-metastatic or metastatic; tumor grades ranged from I to III. Detailed pathology data are available from the authors by request.

Identification of the IRX family and cloning via RACE (rapid amplification of cDNA ends) PCR

To expand our studies of homeobox genes into the human breast, we searched the dbEST database (Boguski et al. 1993) and identified a breast-derived partial cDNA containing a novel homeobox [Expressed Sequence Tag (EST); identification: 152453; GenBank accession: R46202, R46296]. This partial cDNA clone was obtained through the IMAGE consortium and found to be lacking both 5' and 3' ends upon sequencing.

To clone the full-length cDNA corresponding to the IMAGE clone 152453, oligonucleotide primers were designed to the IMAGE clone homeobox and used in RACE polymerase chain reaction (PCR). Primer sequences were: sense "F1": 5'-GCCACGGCTACCCTCAAGGCCTGGCT-3'; antisense "R1": -5'-AGGCCGGCGCGCGTTGNCGAACCA-3'. Template was an adaptor-ligated breast cDNA library (Marathon-ready, Clontech). The library-specific adaptor primer "AP1" was used with "F1" and "R1" in RACE PCR as recommended to generate 3' and 5' ends, respectively.

Fig. 1 **A** *IRX* homeobox alignment. *Asterisks* denote pairwise differences; *periods* denote identities. The position of the "R1" PCR primer is noted. **B** IRX hexapeptide-like and homeodomain amino acid sequences aligned with relevant portions of araucan (*ara*), caupolican (*caup*) and mirror (*mrr*). *Dashes* indicate gaps. *Vertical lines* show pairwise identity between Antennapedia (*Antp*) and mirror and between araucan and IRX-2a. *Plus signs* below the Iroquois-class consensus designate amino acids 100% conserved. The position of the "R1" PCR primer is noted. **C** Northern hybridization: gene-specific pIRX-2 (EK0.5) probe. *Lane 1* represents all normal (*NORM*) samples (*n*=11). *Lane 2* represents a fibroadenoma (*FA*). *Lanes 3* (representing three tumors) *and 4* (representing nine tumors) depict transcript patterns observed in infiltrating ductal carcinomas (*IDC*). *Lanes 5* (representing one tumor) *and 6* (representing two tumors) depict transcript patterns observed in three infiltrating lobular carcinomas (*ILC*). Control hybridization using a probe for the L7 ribosomal protein mRNA was used to assess loading and is shown below. **D** Northern hybridization: homeobox-containing IMAGE probe. *Lane 1* represents all normal samples tested (*n*=7). *Lane 2* represents all tumors tested (*IDC: n*=4/*ILC: n*=1). The small transcript (~1.7 kb) detected by this probe is probably derived from a related *IRX* gene but the possibility of additional transcripts derived from *IRX-2* cannot be ruled out. Control hybridization using a probe for the L7 ribosomal protein mRNA is shown below. **E** Schematic diagram of the *IRX-2a* cDNA and protein. Extent and locations of probes used are shown above the RACE products and IMAGE clone. Locations of the F1 and R1 primers are shown by *arrows*. Amino acid motifs are noted by *color*. **F** *IRX-2a*-translated protein. Motifs noted according to Fig. 1E. **G** Cysteine-rich motif found in some Iroquois-class proteins. **H** Acidic region (partial). **I** IRO box (extended two amino acids to include a conserved alanine). Annotations for **G**, **H** and **I** are as for **B**

While screening 5' and 3' RACE PCR products for cDNAs corresponding to the IMAGE clone, we isolated fragments of transcripts derived from four additional IRX genes. Nucleotide sequence data were immediately deposited in the GenBank sequence database under the following accession numbers: IMAGE clone 152453 (U90309); *IRX-2a* (U90304); *IRX-1* (one isolate) (U90308); *IRX-3* (two identical isolates) (U90305); *IRX-4* (one isolate) (U90306); and *IRX-5* (one isolate) (U90307).

RNA isolation

Mammary tissue was ground in liquid nitrogen to a fine powder. Total RNA was isolated using the Purescript system followed by an additional purification by column chromatography (Qiagen). Total RNA from human uterus, kidney, salivary gland and lung was purchased from Clontech.

Hybridizations

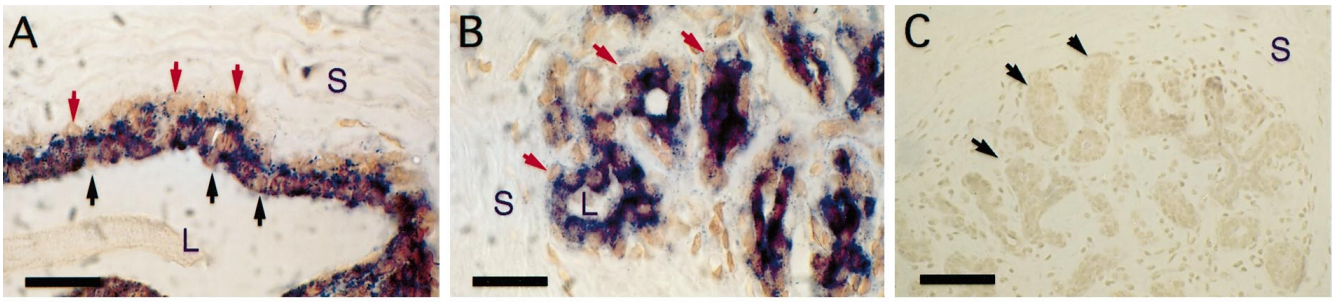
Probe preparation and Northern hybridizations were performed as described (Friedmann and Daniel 1996). The location and extent of probes are depicted in Fig. 1E. In situ hybridization was performed as described (Friedmann and Daniel 1996) using 500 ng/ml antisense or sense pIRX-2 (EK0.5) riboprobes with an additional RNAse A treatment [5 µg/ml RNAse A in 10 mM TRIS, pH 7.5, 1 mM ethylenediaminetetraacetic acid (EDTA), 500 mM NaCl, 37°C, 15 min] between the first and second stringency washes to unequivocally ensure gene-specific hybridization. In control hybridizations, the pIRX-2 (EK0.5) probe does not cross-hybridize with any of the other cloned IRX genes at high stringency (final washes 2×30 min, 0.1×SSC; 0.1%SDS at 65°C); the homeobox-containing IMAGE probe cross-hybridizes with all of the cloned IRX genes at this stringency. Northern hybridizations using the IMAGE probe were done at "unusually high stringency" to obtain gene-specific results (final washes 2×30 min, 0.1×SSPE; 0.1% SDS; 15% formamide at 70°C).

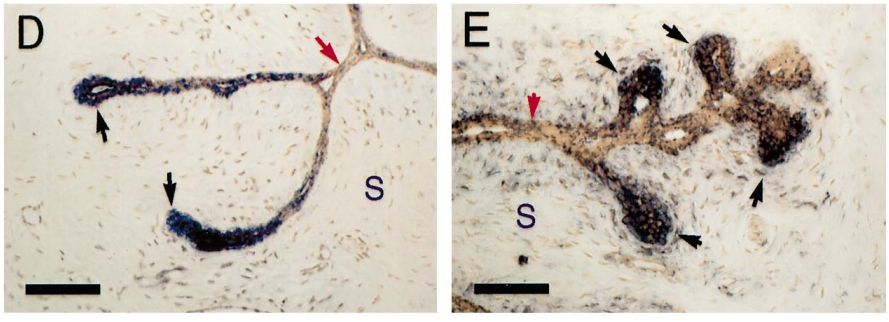
Results and discussion

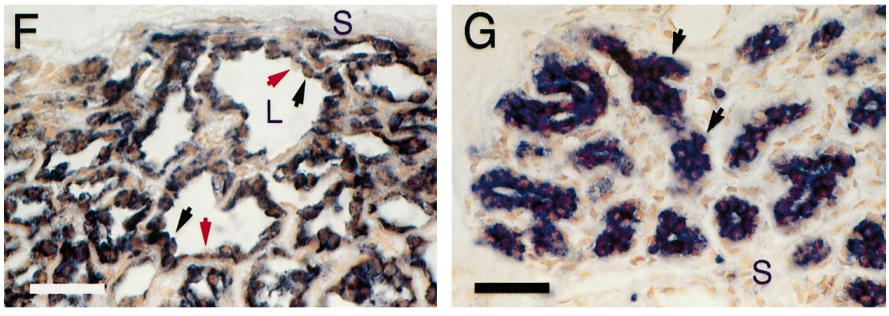
To expand our studies of mammary-associated homeobox genes into the human breast, we searched the dbEST database (Boguski et al. 1993) and identified a breast-derived partial cDNA containing a novel homeobox (obtained through the IMAGE consortium; identification: 152453; GenBank accession: R46202, R46296).

While screening 5' and 3' RACE PCR products for cDNAs corresponding to the IMAGE clone, we isolated fragments of transcripts derived from four additional IRX genes (Fig. 1A,B) giving presumptive evidence for expression of at least five different IRX genes in the adult human breast. To our knowledge, this is the first report of homeobox gene expression of any kind in the human breast. Homologs of this gene family were subsequently identified in *Drosophila* (the Iroquois complex genes) (Gomez-Skarmeta et al. 1996; McNeill et al. 1997) and shown to be required for proper neural patterning. In addition, homologs were recently identified in both mouse and *Xenopus* (Bosse et al. 1997; Bellefroid et al. 1998; Gomez-Skarmeta et al. 1998) and shown to function in the development of the vertebrate nervous system.

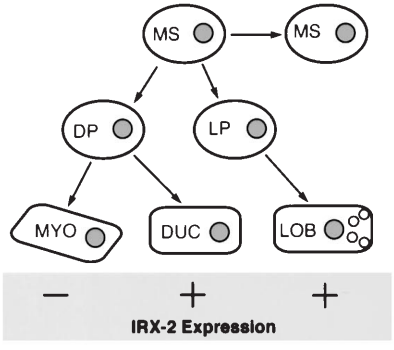
BLAST program database searches (Altschul et al. 1990) showed the IRX homeodomains to be ~90% iden-



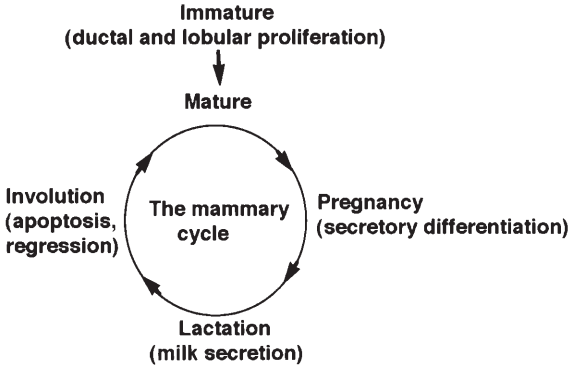


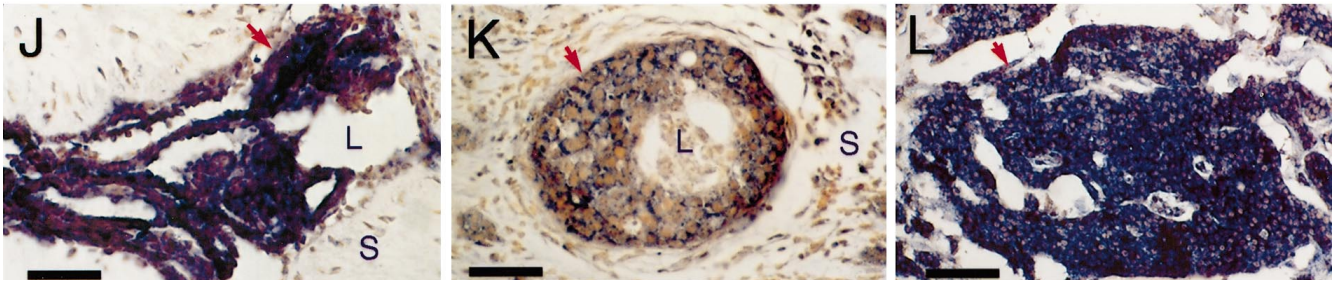


H Mammary Epithelial Cell Differentiation



I Phases of Mammary Gland Development





tical to the Iroquois-class homeodomains encoded by the *Drosophila* genes *araucan* (*ara*), *caupolican* (*caup*) and *mirror* (*mrr*) and between 92% and 93% identical to one another (Fig. 1B) (Gomez-Skarmeta et al. 1996; McNeill et al. 1997). The designation *IRX-2* was ultimately assigned to the gene corresponding to the IMAGE cDNA. Each IRX protein contains a hexapeptide-like motif (Fig. 1B) (Chang et al. 1995; Chan et al. 1996). Thus far, only IRX-3 shows a cysteine-rich motif (Fig. 1G).

Northern hybridization using the gene-specific pIRX-2 (EK0.5) probe (which lacks the homeobox) against normal (*n*=11), benign fibroadenoma (FA) (*n*=1), infiltrating ductal carcinoma (IDCs) (*n*=12) and infiltrating lobular carcinoma (ILCs) (*n*=3) RNAs detected two main transcripts at 2.6 kb and 3.9 kb, designated *IRX-2a* and *IRX-2b*, respectively (representative samples are shown in Fig. 1C). Interestingly, unusually high stringency hybridizations using the IMAGE probe (which contains the homeobox) on normal and tumor samples identified what appeared to be only the single 2.6-kb *IRX-2a* transcript. Since the homeobox-containing IMAGE probe does not hybridize with the 3.9-kb *IRX-2*b transcript, these data suggest that this transcript does not contain a closely related homeobox (Fig. 1D). A similar set of alternatively spliced trancripts both with and without a homeobox has been demonstrated for the mouse *HoxA1* gene (LaRosa and Gudas 1988). Reliable quantitation is not possible using primary tissue samples due to gross variation in the epithelial content of each tissue (evident upon histological analysis) and the unavoidable contamination of tumor tissue with various amounts of normal tissue at the surgical margins. Therefore, we decline to speculate at this time whether *IRX-2* might be misexpressed in some tumors. The question of misregulation of IRX genes will be better approached using microdissection and RNA analysis techniques recently developed for this purpose (Bonner et al. 1997).

To determine whether *IRX-2* expression was mammary specific in the adult, we also conducted Northern analysis on RNA from adult human lung, uterus, salivary gland and kidney using the gene-specific pIRX-2 (EK0.5) probe (data not shown). *IRX-2* messages were weak but detectable in all four non-mammary samples, indicating that expression in the adult is not breast specific.

Cloned, overlapping 5' (*n*=4) and 3' (*n*=3) RACE products comprise a 1.8-kb *IRX-2a* cDNA (exclusive of the polyA tail) that contains a complete open reading frame for the 417-amino acid IRX-2a protein (Fig. 1E,F). In addition to the homeodomain and the hexapeptide-like sequences, the translated cDNA shows acidic and poly-proline regions, and an IRO box (Bürglin 1997) (Fig. 1F–I). Attempts to isolate an *IRX-2b* cDNA via RACE PCR or standard phage-based cDNA library screens were unsuccessful, perhaps due to poor representation resulting from a high G+C content as is observed in the *IRX-2a* cDNA (60–90%, window size=50 bp).

IRX-2 expression was investigated by in situ hybridization using the pIRX-2 (EK0.5) probe against normal breast tissue (*n*=8). In mature tissue, lumenal epithelium of ducts (Fig. 2A) and alveoli (Fig. 2B) express *IRX-2* as evidenced by accumulation of blue-black stain in the cytoplasm of these cells; myoepithelium, adipose stroma, and a small subpopulation of ductal epithelial cells (Fig. 2A) do not express. Sense-strand probe shows no hybridization (Fig. 2C). During the proliferative phase of development, immature tissue shows concentrated expression in terminal end buds and terminal lobules (Fig. 2D,E) becoming reduced in differentiated subtending ducts. During the cyclical phase of mammary development, in lactating tissue (Fig. 2F), ~18% of alveolar epithelial cells show undetectable or reduced expression (cf. Fig. 2B). Uniform expression is re-established in late-stage involuting tissue (Fig. 2G). Tissue from pregnant and early involuting patients was unavailable for study.

Evidence for regulated expression of *IRX-2* is threefold. First, as summarized in Fig. 2H, *IRX-2* is differentially expressed in discreet epithelial cell lineages, being undetectable in differentiated myoepithelial cells but

Fig. 2 Expression of *IRX-2* transcripts in normal development and neoplasia. In situ hybridization using the gene-specific pIRX-2 (EK0.5) probe (detecting both *IRX-2a* and *IRX-2b* transcripts). Expression is evident by accumulation of a blue-black precipitate stain. Ductal and alveolar lumens (*L*) and adipose stroma (*S*) are identified when possible. **A** Major duct showing staining in lumenal epithelial cells. *Red arrows* indicate examples of unstained myoepithelial cells within the myoepithelial cell layer; *black arrows* indicate occasional unstained lumenal epithelial cells. Identification of myoepithelial cells is based on well-established morphological and positional criteria as well as comparison with published immunohistochemical studies using these tissues (Daniel and Silberstein 1987; Russo and Russo 1987; Taylor-Papadimitriou and Lane 1987; Silberstein et al. 1997). Bar 27 μm. **B** Alveoli. Epithelial cells are uniformly stained. *Red arrows* indicate unstained myoepithelial cells. **C** Sense control probe showing a completely unstained lobule. *Black arrows* indicate alveoli. **D** Terminal end buds (*black arrows*). Staining decreases in the subtending duct (*red arrow*). **E** Terminal lobule. *Black arrows* indicate developing alveolar buds which stain heavily for *IRX-2* transcripts. Staining is reduced in the subtending duct (*red arrow*). **F** Lactating tissue in which an increased proportion of epithelial cells (~18%) stain poorly for *IRX-2* transcripts. *Red arrows* indicate unstained epithelium; *black arrows* indicate strongly staining epithelium. **G** Late-stage involuting tissue (not yet completely remodeled). *Black arrows* indicate alveoli in which uniform *IRX-2* expression is re-established in all epithelial cells. **H** A current model for mammary epithelial cell differentiation (Smith 1996) and summary of *IRX-2* expression in differentiated cell types (*MS* mammary stem cell, *DP* ductal progenitor cell, *LP* lobule-alveolar progenitor cell, *MYO* myoepithelial cell, *DUC* differentiated ductal cell, *LOB* differentiated lobule-alveolar cell). The pattern of *IRX-2* expression in each differentiated cell type is shown below. **I** Summary of major phases of mammary gland development. *IRX-2* expression patterns in four of the five developmental phases are represented in panels **A–G** demonstrating changes in *IRX-2* expression through the mammary cycle. **J** Fibroadenoma. *Red arrow* identifies the characteristic network of epithelial cells within the duct. **K** Infiltrating ductal carcinoma. *Red arrow* indicates neoplastic epithelial cells filling the ductal lumen. **L** Infiltrating lobular carcinoma (*ILC*) (*red arrow*). An entire lobule is shown in which histotypic structure is lost (cf. **G**). Hybridizations using the IMAGE probe (which likely detects all five known IRX genes at this stringency) were qualitatively similar to those shown above (data not shown). *Bars* 27 μm (**A, B**), 220 μm (**C–E**), 70 μm (**F, G, J**), 80 μm (**K**), 220 μm (**L**)

readily detected in lumenal ductal and more highly expressed in alveolar epithelial cells. Second, a small subpopulation of lumenal ductal epithelial cells do not express *IRX-2* (Fig. 2A). Finally, with reference to Fig. 2I, there are distinct changes in *IRX-2* expression during different phases of mammary gland development, with concentrated *IRX-2* expression in terminal structures in immature tissue, uniform expression in mature and late-stage involuting tissue, and reduced expression in ~18% of alveolar epithelial cells during lactation.

In tumors (FA, *n*=1; IDC, *n*=7; ILC, *n*=3), *IRX-2* expression is maintained (Fig. 2J–L) regardless of tumor type, grade, receptor status, or metastatic state. In contrast, expression of 9 of 11 normally expressed mouse homeobox genes is lost in neoplastic progression (Hox complex genes B6, B7, C6, C8, D4, D8, D9, D10 and the non-complex gene Msx-2) (Friedmann 1995). Therefore, *IRX-2* may serve as an excellent marker for mammary epithelial cell identity during tumor progression since it is observed in the epithelial component of even high-grade metastatic tumors.

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References

Altschul SF, Gish W, Miller W, Meyers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Bellefroid EJ, Kobbe A, Gruss P, Pieler T, Gurdon JB, Papalopulu N (1998) *Xiro3* encodes a *Xenopus* homolog of the *Drosophila* Iroquois genes and functions in neural specification. EMBO J 17:191–203

Boguski MS, Lowe TM, Tolstoshev CM (1993) dbEST – database for "expressed sequence tags". Nature Genet 4:332–333

Bonner RF, Emmert-Buck M, Cole K, Pohida T, Chuaqui R, Goldstein S, Liotta LA (1997) Laser capture microdissection: molecular analysis of tissue. Science 278:1481

Bosse A, Zülch A, Becker M, Torres M, Gomez-Skarmeta JL, Modolell J, Gruss P (1997) Identification of the vertebrate Iroquois homeobox gene family with overlapping expression during early development of the nervous system. Mech Dev 69:169–181

Bürglin TR (1997) Analysis of TALE superclass homeobox genes (MEIS, PBC, KNOX, Iroquois, TGIF) reveals a novel domain conserved between plants and animals. Nucl Acids Res 25: 4173–4180

Carpenter EM, Goddard JM, Davis AP, Nguyen TP, Capecchi MR (1997) Targeted disruption of Hoxd-10 affects mouse hindlimb development. Development 124:4505–4514

Chan SK, Popperl H, Krumlauf R, Mann RS (1996) An extradenticle-induced conformational change in a HOX protein overcomes an inhibitory function of the conserved hexapeptide motif. EMBO J 15:2476–248

Chang CP, Shen WF, Rozenfeld S, Lawrence HJ, Largman C, Cleary ML (1995) Pbx proteins display hexapeptide-dependent cooperative DNA binding with a subset of Hox proteins. Genes Dev 9:663–674

Chepko G, Smith GH (1997) Three division-competent, structurally-distinct cell populations contribute to murine mammary epithelial renewal. Tissue Cell 29:239–253

Daniel CW, Silberstein GB (1987) Postnatal development of the rodent mammary gland. In: Neville MC, Daniel CW (eds) The mammary gland development, regulation, and function. Plenum Press, New York, pp 3–36

Friedmann Y (1995) Expression and developmental role of homeobox containing genes during mouse mammary gland morphogenesis. Ph.D. Thesis. University of California, Santa Cruz

Friedmann Y, Daniel CW (1996) Regulated expression of homeobox genes *Msx-1* and *Msx-2* in mouse mammary gland development suggests a role in hormone action and epithelial-stromal interactions. Dev Biol 177:347–355

Friedmann Y, Daniel CA, Strickland P, Daniel CW (1994) *Hox* genes in normal and neoplastic mouse mammary gland. Cancer Res 54:5981–5985

Gomez-Skarmeta JL, Diez del Corral R, de la Calle-Mustienes E, Ferres-Marco D, Modolell J (1996) *araucan* and *caupolican*, two members of the novel Iroquois complex, encode homeoproteins that control proneural and vein-forming genes. Cell 85:95–105

Gomez-Skarmeta JL, Glavic A, de la Calle-Mustienes E, Modolell J, Mayor R (1998) *Xiro*, a *Xenopus* homolog of the *Drosophila* Iroquois complex genes, controls development at the neural plate. EMBO J 17:181–190

LaRosa GJ, Gudas LJ (1988) Early retinoic acid-induced F9 teratocarcinoma stem cell gene ERA-1: alternate splicing creates transcripts for a homeobox-containing protein and one lacking the homeobox. Mol Cell Biol 8:3906–3917

Manak JR, Scott MP (1994) A class act: conservation of homeodomain protein functions. Development Suppl:61–71

McNeill H Yang C, Brodsky M, Ungos J, Simon MA (1997) *mirror* encodes a novel PBX-class homeoprotein that functions in the definition of the dorsal-ventral border in the *Drosophila* eye. Genes Dev 11:1073–1082

Phippard DJ, Weber-Hall SJ, Sharpe PT, Naylor MS, Jayatalake H, Maas R, Woo I, Roberts-Clark D, Francis-West PH, Liu YH, Maxson R, Hill RE, Dale TC (1996) Regulation of *Msx-1*, *Msx-2*, *Bmp-2* and *Bmp-4* during foetal and postnatal mammary gland development. Development 122:2729–2737

Russo J, Russo IH (1987) Development of the human mammary gland. In: Neville MC, Daniel CW (eds) The mammary gland development, regulation, and function. Plenum Press, New York, pp 67–93

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning, a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

Silberstein GB, Van Horn K, Strickland P, Roberts CT Jr, Daniel CW (1997) Altered expression of the WT1 Wilms tumor suppressor gene in human breast cancer. Proc Natl Acad Sci USA 94:8132–8137

Smith G (1996) Experimental mammary epithelial morphogenesis in an in vivo model: evidence for distinct cellular progenitors of the ductal and lobular phenotype. Breast Cancer Res Treat 39:21–31

Stuart ET, Yokota Y, Gruss P (1995) *PAX* and *HOX* in neoplasia. Adv Genet 33:255–274

Taylor-Papadimitriou J, Lane EB (1987) Keratin expression in the mammary gland. In: Neville MC, Daniel CW (eds) The mammary gland development, regulation, and function. Plenum Press, New York, pp 181–216

Thesleff I, Vaahtokari A, Partanen AM (1995) Regulation of organogenesis. Common molecular mechanisms regulating the development of teeth and other organs. Int J Dev Biol 39:35–50