Cloning and Expression of Bovine BRCA1

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Abstract: Breast ovarian cancer susceptibility (BRCA1) proteins appear to be involved in cell cycle regulation. DNA repair or genome integrity and induction of apoptosis in a variety of cells from humans and laboratory animals. The BRCA gene and protein have not been identified in cattle. The pattern of BRCA1 gene expression during normal mammary gland development and involution has not been examined in detail in any mammalian species. Therefore, the purpose of the present study was to clone the BRCA1 gene in Holstein dairy cattle and determine if the BRCA1 gene is differentially expressed through various stages of mammary gland development. We also localized immunoreactive BRCA1 protein in bovine mammary cells and milk fat globule membrane. Bovine BRCA1 cDNA was highly conserved to the human. Five hundred base pairs of exon 11 (+3384/ +3866, human BRCA1 cDNA position) and the C-terminus 1 kb were identical to the human. Seven hundred base pairs of the N-terminus, which contain two ring domains, showed 90% homology to human BRCA1. In bovine tissues, the degree of BRCA1 gene expression, from highest to lowest, was as follows: liver, spleen, mammary tissues and kidney. The mammary tissues of early pregnancy heifers (5 months) showed much higher mammary gland BRCA1 mRNA expression than other mammary developmental stages. BRCA1 expression declined during the remainder of pregnancy and remained low for lactating cows. However, its expression increased when involution proceeded. Immunohistochemical studies showed that immunoreactive BRCA1 was localized in the nucleus and cytoplasm of mammary epithelial cells from lactating cows. It was not present in myoepithelial cells. The protein was also localized in the milk fat globule membranes. Our data suggest that BRCA1 is involved in bovine mammary gland development and/or differentiation, is specifically localized in secretory epithelial cells and is likely a secreted protein during normal lactation.

Key words: BRCA1, mammary, development, gene expression, cow, cells, involution

Introduction
Breast ovarian cancer susceptibility (BRCA1) genes and their expressed proteins are currently being studied in human cells and tissues, as well as laboratory animals (Chen et al., 1998; Futreal et al., 1994; Hall et al., 1990; Jensen et al., 1996; Marquis et al., 1996; Milki et al., 1994; Paterson, 1998). These proteins appear to be involved in cell cycle regulation; thereby influencing tissue growth and/or differentiation (Chen et al., 1998; Marquis et al., 1996; Rajan et al., 1996; Rutfner and Verma, 1997). They have also been implicated in DNA repair or genome integrity and induction of apoptosis in a variety of cells (Paterson, 1998; Shao et al., 1996). Moreover, the BRCA1 gene has been identified as a tumor suppressor gene (Paterson, 1998). The gene is mutated in sporadic breast cancer and there are also inherited mutant forms of the gene that show a familial predisposition to cancer (Futreal et al., 1994; Hall et al., 1990; Milki et al., 1994).

The BRCA1 gene encodes a 190-220 kDa protein consisting of 1,853 amino acids (Chen et al., 1996; Paterson, 1998). The protein has sequence homology and biochemical analogy to the grain protein family (Jensen et al., 1996). Both BRCA1 and the grainins, are localized in secretory vesicles and are secreted by a regulated pathway. They are posttranslationally glycosylated, and are responsive to hormones (Romagnolo et al., 1998).

Two putative nuclear localization signals and a potential ring-finger motif suggest that the BRCA1 protein is a nuclear protein. Chen et al., 1996 showed that BRCA1 was a nuclear phospho-protein in normal and non-breast ovarian cancer cells, while in breast/ovarian cancer cells BRCA1 was exclusively localized in the cytoplasm. They suggested that abnormalities of nuclear transport in sporadic tumors might influence BRCA1 cellular localization. Scully et al., 1998; Rutfner and Verma, 1997, however, reported that BRCA1 was predominantly localized in the nuclei of normal as well as breast/ovarian cancer cells.

It is very difficult to obtain non-malignant or “normal” human breast tissue from females over various stages of mammary development, lactation and involution for studies focusing on differential gene expression. Therefore, the pattern of BRCA1 gene expression in normal mammary tissue, over various stages of development, has not been examined in detail. Cattle, however, are useful for studies that are designed to describe changes in gene expression throughout various physiological states. They are convenient for multiple tissue biopsy and blood sampling. Furthermore, much of what we know about mammary gland biology has been derived from cattle and other ruminants. The purpose of the present study was to clone the BRCA1 gene in Holstein dairy cattle and determine if the BRCA1 gene is differentially expressed through various developmental stages of the mammary gland. We also localized immunoreactive BRCA1 protein in mammary cells and milk fat globule membrane.

Materials and Methods
Cloning and Sequencing of Bovine BRCA1: Total RNA was isolated from 0.5 g of mammary tissue of a non-lactating cow obtained from the slaughter house. Tissue was homogenized by a Polytron homogenizer in Tris buffer (Molecular Research Center) according to the manufacturer's recommendations. Total RNA (1 μg) was used for reverse transcription (RT) with a Cycle Kit (Invitrogen). RT procedures were according to manufacturer's recommendations, and 1/5 of the RT product (4 μg) was used for PCR amplification. The PCR steps were performed as follows: 96 °C for 3 min, then 30 cycles of amplification (96 °C × 30 sec, 52 °C × 30 sec, 68 °C × 4 min), ending with 85 °C × 8 min. Advanced Taq polymerase (Gentech) was used for amplification. The amplified products, 6.6 kb and 2.1 kb, were cloned into Topo-XL Cloning Kit (Invitrogen). The procedures used were according to the manufacturer's instruction. The cloned elements were sequenced at the Cornell University sequencing facility. All primers used are shown in Table 1.

Northern blot analysis: For Northern blot analysis, 15 μg of total RNA was mixed with formaldehyde loading buffer (40 mM Mops pH 7, 10 mM sodium acetate, 1 mM EDTA, 50% formamide, 5% formaldehyde), and loaded on a denatured agarose gel (40 mM Mops pH 7, 10 mM sodium acetate, 1 mM EDTA, 5% formaldehyde, 1% agarose). After electrophoresis, samples were transferred onto nylon membranes (Gene Screen; Dupont New England Nuclear) and UV cross-linked. Prehybridization, hybridization, and washing steps were performed, as described by Sambrook et al., 1989.

Briefly, the 6' region of BRCA1 cDNA, 1.2 kb, was used as a
Table 1: Primers used to amplify various regions of bovine
BRCA1 cDNA by PCR

<table>
<thead>
<tr>
<th>Name of Primer pair*</th>
<th>Position†</th>
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<tr>
<td>851045/0414A</td>
<td>+85/+6669</td>
</tr>
<tr>
<td>Y15/3Y23A</td>
<td>+225/+809</td>
</tr>
<tr>
<td>851045/0412A</td>
<td>+85/+809</td>
</tr>
<tr>
<td>7925/2142A</td>
<td>+782/+4214</td>
</tr>
<tr>
<td>LAlS/8265A</td>
<td>+3384/+3847</td>
</tr>
<tr>
<td>LAlS/9265A</td>
<td>+2767/+3847</td>
</tr>
<tr>
<td>RG25/0611A</td>
<td>+4681/+5584</td>
</tr>
</tbody>
</table>

*All primers were 20 nucleotides in length. 5 for sense strand and
A for antisense strand. 1Primer position is based on the human
BRCA1 cDNA sequence NID is g555921

template to make a cDNA probe. The cDNA probe was labeled with
P-32-DATP by a random primer DNA labeling kit (Life Technologies, Inc.)
Prehybridization was carried out in hybridization buffer (6X SSPE, 6X Denhardt's solution, 0.1% SDS, 50% formamide, and 100 µg/ml Salmon sperm DNA) for 1 hour
and 1X10^6 cpm/ml of the labeled probe were added and incubated
over night at 42 °C. The membrane was washed twice with 2x
SSPE/0.1% SDS for 30 min at 65 °C, and 1x SSPE/0.1% SDS for
1 hour at 65 °C prior to autoradiography exposure.

Western Blot Analysis: Bovine mammary tissue from a lactating
cow (5 g) was homogenized in a Polytron with 2 ml of 0.25 M
sucrose containing 0.1 M Tris-HCl (pH 7.4) and the following
protease inhibitors: 1mM PMSF, 25 µg/ml leupeptin, 25 µg/ml
apotinin, 1 mM benzamidine and 10 µg/ml soybean trypsin
inhibitor (Sigma Chemical Co.). The homogenate was then
centrifuged at 36,500 rpm for 20 min at 4 °C. After
centrifugation, the supernatant was collected and quantified for
protein concentration using the Bradford method (BioRad).
The supernatant (120 µg) was run on a 5% SDS-PAGE, transfered
to a PVDF membrane, the blot was blocked with phosphate-buffered saline (PBS, pH 7.2) containing 3% BSA and 0.05% Tween-20.
BRCA1 immunoblots were performed using the BRCA1-BF7 monoclonal
antibody (Genetex), or BRCA1-A6S (Oncogene Research), diluted
1: 50 in 1% BSA in PBS. Binding was visualized by incubating the membrane with a horseradish peroxidase conjugated rabbit anti-mouse antibody (Sigma). The
immunocomplexes were detected by color development with 4-
chloro-1-naphthol (Sigma) and hydrogen peroxide, or LumiGLO
chemiluminescent substrate (KPL).

Distribution of BRCA1 in bovine tissues
Ribonuclease Protection Assay (RPA): The expression patterns of
BRCA1 mRNA in various bovine tissues and in mammary tissue
across various physiological stages and involution (see below)
were measured by RPA and a Multi-NPA hybridization procedure
(Ambion). Liver, spleen, kidney, and mammary gland (5g of each
tissue) were isolated from three non pregnant cows in the first
and seventh month of lactation. Briefly, total RNA was extracted
from liver spleen kidney and mammary tissue using Tris reagent
(Molecular Research Center), as described by the supplier. The
template to generate a ribo-probe was prepared by PCR from
bovine BRCA1 cDNA. BRCA1 primer pair, forward (+85/+104):
5'tctgctctggtaaaggctcttgca 3' and backward (+640/+620):
5'attagtgagcataaagtagtgatctGCCTgtcg 3' was used for
preparation of template. The reverse primer contains the SP6 RNA
polymerase binding site (underlined in the above sequence) to
make antisense ribo-probe. A 500 nt biotin labeled antisense ribo-
probe was synthesized by Maxi Script (Ambion) according to the
manufacturer's recommendations. The synthesized probe was gel
purified and hybridized with 15 µg of total RNA to protect a
fragment of the expected length. After nuclease digestion, the
protected fragment was electrophoresed and transferred to a
nylon membrane by electro-transfer. Protected fragments were
detected by Ambion's Bright star bio-detection kit. BRCA1 mRNA
was quantified by Photo Image IS10000 with arbitrary units
corrected for the expression of the control, actin mRNA
(BRCA1/Actin).

Differential expression of BRCA1 in mammary tissue
Mammary biopsy: Mammary tissue was obtained by biopsy from
conscious female Holstein cattle in the following physiological
stages (primiparous and three and eight months pregnant;
multiparous and lactating for 3-6 months (early lactation);
multiparous and lactating for 6-8 months (mid lactation);
multiparous and lactating for 9-11 months (late lactation); and
multiparous and undergoing mammary involution (three, six, and
eight weeks). Three animals were biopsied per each developmental
stage.

Animals were brought into a surgery suite and the point of tissue
excision was anesthetized with lidocaine. Five ml lidocaine was also
injected around the excision site. Approximately 10 grams of
tissue was removed containing parenchymal elements. Blood
vessels were cauterized or sutured to prevent further bleeding after
biopsy removal. The excision site was closed with self-
dissolving suture. Animals were given an injection of penicillin
and returned to the Cornell University dairy farm.

Total RNA was extracted from the mammary biopsy samples
following the procedures described above. The expression
patterns of BRCA1 mRNA in mammary tissue across various
physiological stages and involution were measured by RPA and a
Multi-NPA hybridization procedure (Ambion) as described above.

Identification of BRCA1 in mammary tissue, milk fat globule
membrane and MCF-7 breast cancer cells
Mammary tissue: Mammary tissue (25 grams) was obtained from
a lactating Holstein cow at slaughter. The tissue was cut into 10
mm3 sections, fixed with Bouin's fixative and embedded in
paraffin and then sectioned. After cutting serial sections (6 µm),
slides were mounted on plain glass slides. Slides were deparaffinized
through a series of xylene baths and then rehydrated. Finally,
slides were placed in a Microprobe holder. The slides were dipped
and blotted one time in 0.5% hydrogen peroxide in methanol,
and then dipped and left for 10 minutes to block endogenous
peroxidase activity. Slides were incubated with normal blocking
serum (goat) for 10 min at room temperature in a humid chamber
and then blotted. The slides were incubated with 1: 500, 1:200
affinity purified rabbit polyclonal antibody to BRCA1 (B-20, Santa
Cruz Biotechnology) or 1: 50, 1:200 mouse monoclonal antibody
to BRCA1 (Oncogene Science) in a humid chamber for 2 hours at
37 °C. Non-immune serum was used for control slides. After
incubation, biotinylated secondary anti-rabbit or anti-mouse IgG
antibody (2µg/ml) were applied to slides for 20 min at room
temperature in a humid chamber. After incubation and washing
slides were incubated again with streptavidin-peroxidase conjugate
for 10 min at room temperature in a humid chamber. Slides were
removed from the Microprobe holder and incubated ( 30 S) with
chromogen/substrate solution (2µ/ml) at room temperature. Slides
were then washed in tap water and counterstained with
Gill's #1 hematoxylin for 10-30 seconds. The slides were washed
in tap water and blotted. Permount was added to each slide and
the cover slips were attached. Staining was visualized using a
binocular microscope.

Isolated MFGM: Milk fat globule membranes (MFGM) were
prepared as described by Spitsberg et al.,1997 with minor
modifications. Milk was obtained from a healthy mastitis free cow
in early lactation. Cream was isolated by centrifugation of milk at 3,000 x g for 50 min. It was then suspended in two volumes of
50 mMol/L Tris-HCl, pH 7.5, containing 0.15 mMol/L NaCl (TBS),
and homogenized in a Waring blender for 1 min. The homogenate
was centrifuged at 100,000 x g for 90 min at 4 °C. The MFGM
pellet was washed once in TBS and resuspended in a small volume
of TBS, followed by recentrifugation at 100,000 x g for 90 min and
stored in a -70 °C freezer. No somatic cells were found in the
MFGM, as determined by light microscopy.
Chung and Gorewit: BRCA1 Expression

3.7% formalin in PBS (pH 7.4) for 30 min, they were washed in PBS, 3x for 5 min. The slides were treated with PBS + 0.1% Triton X-100 for 5 min to permeabilize the membranes and the slides were dried. Immunostaining was carried out as described above for monomeric tissue.

MCF-7 breast cancer cells: BRCA1 is expressed in MCF-7 human breast cancer cells (Patakk, 1999). Therefore, we isolated an MCF-7 total cell lysate, as a positive control for experiments identifying BRCA1 in bovine tissues and MFGR. MCF-7 cells were cultured and protein extracts were prepared using cell lysis buffer (Promega). MFGR was prepared as described above. MCF-7 protein extracts (100 µg) and MFGR (200 µg) were electrophoresed on a 5% SDS-PAGE, transferred to a PVDF membrane. The blot was blocked with phosphate-buffered saline (PBS, pH 7.2) containing 3% BSA and 0.05% Tween-20. The BRCA1 immunoblot was performed using BRCA1-7F3 monoclonal antibody (Oncogene), BRCA1-Ab3 (Covance), 1-20 (Santa Cruz biotechnology) diluted 1:50 in 1% BSA in PBS. Binding was visualized by reacting with a horseradish peroxidase conjugated secondary antibody (Sigma). The immunocomplexes were detected by peroxide; or Lumidio chemiluminescent substrate (KPL).

Results and Discussion

Cloning and sequencing of bovine BRCA1: RT-PCR was performed with RNA from bovine mammary tissue, using primers derived from exons 3 and 24 of human BRCA1. Two PCR products (Fig. 1) were observed that would have derived from full length BRCA1 (i.e., about 5.6 kb, and 2.1 kb) as in human. Mikl et al., 1994 reported that a breast tissue-derived BRCA1 cDNA clone in which exons 9, 10, 11 were spliced out. These exons are 47, 76, and 3427 nucleotides in length, respectively. The small fragment was considered as exon 11 deletion splicing variant. This expectation was confirmed by northern analysis in which two transcripts were observed: one band around at 7.8 kb and the other one is at 4.4 kb (Fig. 2). Studies to determine whether splicing variants are functionally active are ongoing. Bovine BRCA1 cDNA was highly conserved to the human. Five hundred base pairs of exon 11 (+3384A to +3688, human BRCA1 cDNA position) and the C-terminus 1 kb were identical to the human. Seven hundred base pairs of the N-terminus, which contains two ring domains, showed 90% homology to human BRCA1 (Fig. 3). The sequence of 120 nt splicing junction, beginning at nucleotide 615, was as follows: CAGGATCTCTGC (lower case letters represent the 120 nt that were spliced out). The Southern blot analysis (Fig. 4) showed that size of bovine BRCA1 was quite similar to human's (i.e., 180-220 KDa).

Distribution of BRCA1 in bovine tissues: The distribution of bovine BRCA1 mRNA in spleen, kidney, liver and mammary tissues was analyzed by the RNase protection assay (Fig. 6). A probe spanning exons 2 to B, as described in the methods section, was used for the RPA assay. Tissues were obtained from a cow in early lactation. The level of BRCA1 mRNA expression in all tissues was similar to that found in the mouse. The degree of bovine BRCA1 expression from highest to lowest, was as follows: liver, spleen, mammary tissues and kidney.

Differential expression of BRCA1 in mammary tissue: BRCA1 mRNA expression was examined over developmental stages from bovine mammary tissue by RNase protection assay (Fig. 8). The mammary tissues of early pregnancy heifers (3 months) showed much higher mammary BRCA1 mRNA expression than other mammary developmental stages. BRCA1 expression declined during the remainder of pregnancy and remained low for lactating cows. However, its expression increased when involution proceeded.

Marque et al., 1995; Rajan et al. 1999 showed that BRCA1 is broadly expressed in tissues of the early embryo, and is expressed
Fig. 3: Ring finger domain (N-terminus) of Bovine BRCA1 cDNA Sequence. (A) The nucleotide sequence of bovine BRCA1 corresponds to human BRCA1 exons 2 to 8. The 120 bp splicing point is underlined. (B) Bovine BRCA1 sequence comparison with human and mouse.
Chung and Gorewit: BRCA1 Expression

Fig 4: Western blot analysis of bovine BRCA1. Each lane contains 120 μg of crude extracts prepared from lactating bovine mammary tissue. The extracts were electrophoresed on 5% SDS-PAGE and then transferred to PVDF membranes. Protein was detected using N-terminal BRCA1 monoclonal antibody (BRCA1-8F7).

A)

<table>
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<th>(kDa)</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td></td>
<td>187</td>
<td>118</td>
<td>85</td>
</tr>
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BRCA1

Actin

Fig 5: Tissue distribution of BRCA1 in a lactating cow. (A) Total RNA was isolated from a cow in early lactation and BRCA1 mRNA expression was analyzed by RNase protection assay, using a biotin-labeled antisense BRCA1 probe. (B) Quantification of BRCA1 mRNA expression in (A). Relative BRCA1 mRNA expression to β-actin mRNA expression is shown. Representative autoradiographs are shown in A. Means and standard errors are shown for three separate electrophoretic runs in B.

Fig 6: Bovine mammary gland BRCA1 expression over various developmental stages. (A) RNase protection assay (RPA) was used to quantify bovine BRCA1 mRNA expression. Top and mid-panel are RPA hybridized to the 550 nt, 560 nt bovine BRCA1 antisense riboprobe and 280 nt actin riboprobe, respectively. Each lane contains 1μg of total RNA extracted from indicated developmental stage of bovine mammary tissue. (B) Quantification of BRCA1 mRNA expression showed in (A). Relative BRCA1 mRNA expression to β-actin mRNA expression is shown. EP (Early Pregnancy): 3 months; MP (Mid Pregnancy): 5 months; LP (Late Pregnancy): 6 months; LL (Late Lactation): 0 months; LL (Late Lactation): 3 months; ML (Mid Lactation): 6 months; EL (Early Lactation): 9 months; BLB Involution, and 2nd Involution. Representative radiographs are shown in A. Means and standard errors are shown for three separate electrophoretic runs of three animals at each developmental stage in B.

In an epithelial-specific fashion in the mammary gland of mice. The highest BRCA1 expression was found in the terminal end bud structures which contain rapidly dividing, relatively undifferentiated cell types that give rise to the differentiated mammary epithelial tree during ductal morphogenesis, during puberty and in developing alveoli during pregnancy. Taken together, BRCA1 is considered to regulate cellular proliferation and differentiation.

Differentiation of the mammary epithelial cell is completed in mid-pregnancy in the bovine. Early pregnancy tissue (3 month) showed the highest BRCA1 expression, as expected. The expression of BRCA1 was maintained high until mid-pregnancy and then decreased until late lactation. So, if we consider these expression patterns, BRCA1 may serve as a regulator of cell proliferation in bovine mammary tissue. In other words, through all of pregnancy, BRCA1 may regulate the cell cycle and/or repair DNA. In addition, the interesting point is that BRCA1 expression increased over involution as in mice. The involution period includes a tissue remodeling process with widespread apoptosis taking place. Shao et al., 1990 showed that serum deprivation or calcium ionophores treatment of BRCA1-transfectants resulted in programmed cell death in NHERF3 and MCF-7 cell lines. So far, there is no direct evidence that BRCA1 is directly involved in apoptosis during involution. Nevertheless, further research is needed to answer it clearly. Marques et al., 1996 reported the mammary glands of parous mice that had undergone four weeks of postlactational regression express higher levels of BRCA1 mRNA than the mammary glands of age-matched virgin control mice.
BRCA1 Expression in Mammary Tissues and Tissue Distribution

A) Control (NRS for Ab-3)

B) Ab-3

C) Control (NRS for I-20)

D) I-20

Fig. 7: Localization of BRCA1 in lactating bovine mammary tissue. All slides were processed using the Ab-3 (A, B) and I-20 (C, D) BRCA1 antibodies and immunohistochemistry protocols described in materials and methods. BRCA1 signals were photographed in tissue at 200X (A, C) and at 1,000X (B, D). Note that myoepithelial cells seen with elongated nuclei surrounding alveolar cells are not immunostained.

Murine mammary gland BRCA1 expression varies through all stages of mammary differentiation. It was reported that BRCA1 mRNA expression is very low during lactation in mice (Marquèze et al., 1996; Rajan et al., 1998). Bovine BRCA1 mRNA expression is very similar to that of the mouse. BRCA1 protein expression, however, was quite high during lactation compared to other developmental stages. We feel that the elevated levels of BRCA1 protein seen during lactation are a result of regulatory mechanism(s) involved in translation, or RNA stability.

Identification of BRCA1 in mammary tissue, milk fat globule membrane and MCF-7 breast cancer cells

Mammary tissue: We used two antibodies in immunohistochemistry (IHC) to detect immunoreactive bovine BRCA1 in lactating mammary tissues. Both I-20, and Ab-3 antibodies localized BRCA1 in the nucleus, cytosol, and milk fat globule membrane (MFGM) (Fig. 7). The significance of BRCA1 localization in the MFGM shows that it is most likely a secreted protein. This finding supports the hypothesis for BRCA1 localization in the endoplasmic reticulum (ER)/golgi (Jensen et al., 1998).

Our immunolocalization results showed the staining as very intense in epithelial cells, but not in myoepithelial cells, or fibroblasts (Fig. 7). This implies that the signal is cell specific.
immuno-localization studies gives strong support for the secretion of BRCA1 in lactating tissues. It can be argued that our isolated MFGM might be contaminated from secreted epithelial cells and proteins from dead cells. First, we can not rule out that there was contamination. However, we are sure through our experience that most of cells centrifuged at 3,000 x g for 90 min. This was confirmed under the microscope at the end of the procedure. We do not believe that cell contamination (membrane debris) contributed to our results, because a physiological buffer was used to isolate the MFGM which was isotonic (TBS pH 7.2). We do not believe that isotonic TBS can precipitate free proteins at 100,000 x g for 90 min at 4°C. So, we ruled out the possibility of contamination during MFGM suspension and washing steps. From our data, we believe that bovine BRCA1 is a secreted tumor suppressor protein during lactation. Our next approach is to investigate the molecular mechanisms regulating BRCA1 synthesis and secretion.

Identification of BRCA1 in MCF-7 breast cancer cells: We isolated an MCF-7 total cell lysate isolates, as a positive control for our BRCA1 immuno-histochemistry studies since the protein is expressed in human MCF-7 breast cancer cells. We used three different antibodies for Western blot analysis. The three antibodies, I-20, Ab-3, and BRCA1 17F8, developed from exon 11, were used. Our Western blot results showed that all three antibodies, against BRCA1, were detected in the MCF-7 lysate (Fig. 5). This further suggested that BRCA1 was present in bovine tissue and the MFGM and helps confirm our cloning procedures. Even though our results are limited, with regard to unequivocally proving that BRCA1 is a secreted protein, we speculate that a functional shift of epithelial cells to differentiated secretory alveolar cells allow BRCA1 secretion to occur in response to hormonal influences during lactation.

Bovine BRCA1 cDNA was highly conserved to the human. Five hundred base pairs of exon 11 (+3394/+3890, human BRCA1 cDNA position) and the C-terminus 1 kb were identical to the human. Seven hundred base pairs of the N-terminus, which contains two ring domains, showed 80% homology to human BRCA1. The degree of bovine BRCA1 expression from highest to lowest was as follows: liver, spleen, mammary tissues and kidney.

The mammary tissues of early pregnancy heifers (3 months) showed higher mammary BRCA1 mRNA expression than other mammary developmental stages. BRCA1 expression declined during the remainder of pregnancy and remained low for lactating cows. However, its expression increased when involuted proceeded. Immunohistochemical studies showed that BRCA1 was localized in the nucleus and cytoplasm of mammary epithelial cells from lactating cows. BRCA1 was not found in myoepithelial cells. The protein was also localized in the milk fat globule membranes. Our data suggests that normal bovine mammary tissue contains immunoreactive BRCA1 protein and that the BRCA1 gene is differentially expressed through various stages of mammary development and involution. The protein appears to be a secreted protein in normal mammary tissue, since immunoreactive BRCA1 is present in the milk fat globule membrane. Even though our results are limited, with regard to unequivocally proving that BRCA1 is a secreted protein, we speculate that a functional shift of epithelial cells to differentiated secretory alveolar cells allow BRCA1 secretion to occur in response to hormonal influences during lactation.

Further studies are necessary to determine the role of this protein in bovine mammary gland development and involution.

References

Fig. 8: Identification of BRCA1 in MFGM. All slides were processed using the Ab-3 (A, B) BRCA1 antibody and immuno-histochemistry procedures were as described in materials and methods.

Fig. 9: Western analysis of BRCA1 from MCF-7 cell extracts and MFGM. 100 μg of isolated MCF-7 lysate and 200 μg of MFGM were loaded on 5% SDS PAGE and then transferred on to a PVDF membrane. Protein was detected using Ab-3 (A, 17F8 (B), and I-20 (C) BRCA1 antibodies.

Isolated MFGM. In addition, we observed intense BRCA1 staining in isolated MFGM (Fig. 8). The Ab-3 monoclonal antibody used in

62
Chung and Gorewit: BRCA1 Expression


