Decreased MCF-7 Breast Cancer Cell Proliferation by Serum from a Selected Line of Beef Cattle

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Abstract. One way to combat or treat breast cancer, the most common cancer among women, is to decrease or prevent proliferation of cancerous cells. Many animal species of agricultural importance have been selected for various growth traits, typically through altered proliferative capacity of some cell types. Previous studies in two lines of cattle divergently selected for serum IGF-I concentration have shown that low IGF-I cattle had increased growth rates and high IGF-I cattle had decreased growth rates. Serum from either the high or low IGF-I lines of cattle were administered to MCF-7 breast cancer cell lines and doubling times were determined. The MCF-7 cells treated with serum from cattle with high serum IGF-I concentrations took 26% longer to double than MCF-7 cells treated with serum from cattle with low serum IGF-I concentrations. In conclusion, model systems employing agricultural animals may provide novel insight into mechanisms of cell proliferation.

Insulin-like growth factor-I (IGF-I) enhances cell proliferation, survival, and differentiation. As a key regulator of cell cycle progression, IGF-I also plays an important role in malignant tumor transformation and invasion (1, 2). Extensive study has demonstrated that IGF-I is a mitogenic and antiapoptotic agent for breast epithelial cells in vitro (3) and is crucial for mammary gland development (4). Furthermore, numerous reports have demonstrated a supportive role for IGF-I in breast cancer pathogenesis (5). First, breast cancer cell lines express all of the components required for eliciting a response to IGF-I, and IGF-I is one of the most potent mitogens for breast cancer cells (6). Second, blocking IGF-I action in vitro and in vivo can inhibit breast cancer cell growth (7, 8). Third, an epidemiological study shows that circulating levels of IGF-I predict breast cancer risk (9). Finally, IGF components are expressed in primary breast cancer and high expression of several of the components are associated with poor prognosis (10).

Studies in cattle and other species have shown that increased serum concentrations of IGF-I correlate positively with increased growth and increased marbling or intramuscular fat deposition (11, 12, 13). In contrast, a population of Angus cattle at the Eastern Ohio Resource Development Center (EORDC) has been divergently selected for serum concentrations of insulin-like growth factor I (IGF-I) leading to a group of animals with comparatively higher concentrations of serum IGF-I (high line) compared to a group with lower concentrations of serum IGF-I (low line). The cattle within these two lines exhibit a negative genetic correlation of serum IGF-I with both growth rate (-0.38) and intramuscular fat deposition (-0.53). These correlations imply that cattle from the low line have increased growth rates and increased intramuscular adipocyte fat accrual and that cattle from the high line have lesser growth and marbling (14, 15, 16).

Control of IGF-I concentration is regulated at several sites in the animal. Specifically, most of the serum IGF-I is produced by the liver in response to growth hormone (GH) stimulation. Once in circulation, IGF-I is regulated by insulin-like growth factor binding proteins (IGFBP), which bind IGF-I and inhibit its binding to tissue receptors. No differences in IGFBP levels exist between the two selection lines of cattle (17). This leads to the hypothesis that an unknown factor, in the high IGF-I line co-selected with IGF-I, is causing decreased proliferative capacity. Higher IGF-I is normally associated with increased growth, and, thus, increased proliferation and differentiation.
Numerous growth factors, hormones, and environmental agents regulate the signaling events involved in carcinogenesis. After lung cancer, breast malignancies are the second-leading cause of cancer death in American women (18). Both age and the duration of exposure to endogenous and exogenous estrogens may be one of the best-defined risk factors linked to human breast cancer. For women between 40 and 55 yr of age, breast cancer is the leading cause of death. Approximately 40,000 women die of this breast disease every year, and estimates indicate that more than 180,000 women in the U.S. will be diagnosed with breast cancer every year (1). If the incidence of breast malignancies in women continues to follow current trends, one in 10 women born today will develop a breast malignancy (19, 20).

Basic and clinical research has contributed to an understanding of the factors involved in the development of breast cancer. Genetic factors predominant in the manifestation of breast cancer disease include gene amplifications, gene deletions, point mutations, loss of heterozygosity, chromosomal rearrangements and aneuploidy. Epigenetic modifications have been implicated as one of the most relevant molecular alterations in a variety of human neoplasias (21, 22, 23). Experimental cancer models have demonstrated that the extracellular microenvironment influences tumor formation, the rate of cellular proliferation, the ability of the cancer cells to metastasize and the extent of invasiveness. In cancers, the influences of the microenvironment are mediated in part by paracrine signaling between epithelial cancer cells and the surrounding stromal cells (24, 25). We hypothesize that components in the serum from the high IGF-I line of cattle mimic paracrine signals capable of suppressing the proliferation events characteristic of human breast neoplasms.

Materials and Methods

Selection procedures. Data for this study were taken from an experiment involving divergent selection for serum IGF-I concentration that was initiated in 1989 using 100 spring-calving (50 high line and 50 low line) and 100 fall-calving (50 high line and 50 low line) purebred Angus cows with unknown IGF-I concentrations located at the Eastern Ohio Resource Development Center (EORDC), Belle Valley, OH, USA. Cows from the initial base population were randomly assigned to the selection lines.

Selection procedures and the mating scheme for this experiment have been described previously (2, 16). Briefly, the four bull calves with the highest and the four with the lowest residuals (adjusted for age of calf and age of dam) for IGF-I concentrations were saved each year for breeding within the respective selection lines. Selection was based on the mean IGF-I concentration of three blood samples collected at the same time as for bulls.

Management procedures. Spring-born calves were reared by their dams without creep feed until weaning at approximately 7 mo of age. Following weaning, bull calves were given ad libitum access to a corn-soybean meal based diet plus grass hay (2.3 kg/bull-1·d-1). Heifers born from spring 1989 through fall 1993 were given ad libitum access to nonprotein nitrogen (feed grade urea) treated corn silage, in addition to grass hay in large round bales. Heifers born in spring 1994 and later years were fed a corn-soybean meal diet intended to yield postweaning gains of approximately 75 kg/d. Bulls were fed in a three-sided barn with adjoining exercise lots located at EORDC. Heifers born from spring 1989 through fall 1993 were fed in a drylot with access to an enclosed barn located at the North Appalachian Experimental Watershed (NAEW), Coshocton, OH. Heifers born in spring 1994 and later were fed in a three-sided barn with adjoining exercise lots located at EORDC.

Fall-born calves were weaned at an average age of 140 d and then fed a corn-soybean meal diet formulated to yield gains of approximately 0.9 kg/d, plus grass hay, in drylot for 112 d. Following the 112-d growing period, bull calves remained at EORDC and were managed in the same manner as spring-born bulls. Heifers born during fall 1993 and previously were transported to NAEW and managed in the same manner as spring-born heifers. Heifers born during fall 1994 and later remained at EORDC and were fed the same diet as spring-born heifers.

Serum samples. Approximately 25 ml of blood was collected into sterile 16-mm x 150-mm glass tubes via jugular puncture of each animal. The blood was allowed to clot for 24 h at 4°C. Serum was obtained by centrifugation (1,800 x g for 20 min) and frozen at -20°C until it was assayed.

Radioimmunoassay for insulin-like growth factor I. The RIA for IGF-I concentration was performed at the University of Florida using antiseraum raised against human IGF-I in rabbits (UBK487), following previously described procedures (14).

Proliferation assays. Doubling time cell proliferation assays were employed to evaluate MCF-7 cell proliferation after exposure to serum from high and low IGF-I line cattle for 48 hr. The final IGF-I concentration in the low line media was 9.95ng/ml and the concentration in the high line media was 37.90ng/ml. The doses selected for the in vitro studies were optimal effective doses which were estimated from our published or preliminary data. For the doubling time assay, cells were plated in 24-well plates. After cells attached to the wells, the medium was replaced with 2 mL of fresh culture medium. At time 0, cells were counted. Cells were grown for 3 d and counted every 8 h with a hemacytometer. Experiments were performed twice in four replicate wells. Based on the cell numbers, determined at different time points, a cell proliferation curve was generated. Cell doubling (CD) was calculated using the formula ln (Nj-Ni)/ln 2, where Nj or Ni are the cell numbers at different time points, Tj or Ti (Tj > Ti), in the log growth phase.
of the cells. Doubling time (DT) was obtained by dividing the time interval \((T_f > T_i)\) by CD.

**Results**

MCF-7 cells cultured with FBS, doubled in 7.262±0.363 hr, cells cultured with low IGF-I line sera doubled in 10.489±0.664 hr, and cells cultured in high IGF-I line serum doubled in 14.255±0.818 hr. The MCF-7 cells grown with high IGF-I line serum doubled at a rate 26% slower than MCF-7 cancer cells grown with serum from the low IGF-I line of cattle \((p<0.05)\) (Figure 1).

**Discussion**

This research shows that previously established animal lines can be mined for potential factors that could have therapeutic benefits to human health. The serum from the line of cattle selected for high serum IGF-I concentration resulted in significantly lowered proliferation rates of the MCF-7 breast cancer cell line. These findings suggest that the factor in the serum of the high line cattle is quite potent, as MCF-7 cells are known to have increased proliferative rates with increased IGF-I concentration. Yet, even with a 4 fold higher IGF-I content, the high IGF-I line serum still repressed proliferation.

Recent studies have determined that E-peptides of pro-IGF-I from animal species can alter growth patterns of certain cell types in vitro (26). E-peptides from rainbow trout can suppress anchorage independent colony formation in neuroblastoma cells and hepatoma cells (27, 28, 29). Putative membrane receptors have been determined to have high affinity for pro-IGF-I E-peptides (30).

While the causative factor(s) is unknown, future studies are planned to determine what factors present in the serum have anti-proliferative effects. The results indicate that a factor(s) in the serum is causing the difference in breast cancer cell growth. Further studies employing proteomic analyses are planned to identify the factors underpinning the mechanism of decreased proliferation events in the MCF-7 cell line.

Future research employing these selected cattle to determine if similar results occur with extracts from the muscle tissue are planned.

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**References**


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