The FGFR1 chromosomal locus is amplified in 10% of breast cancer patients [2].

http://www.dnastuff.com/products/NOVL0016_lg.jpg

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Genetic Approach to Generating a Novel Mouse Model of Mammary Tumorigenesis

http://www.sciencedaily.com/releases/2008/08/080815140250.htm

Methods

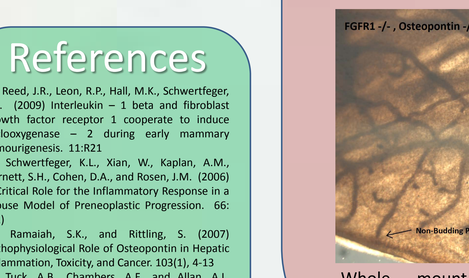
Breeding Scheme

Osteopontin Knockout

FGFR1 +

http://www.clker.com/clipart-mouse-10.html

http://www.clker.com/clipart-simple-cartoon-mouse-1.html



FGFR1 is on a FVB background. The Ostepontin Knockout genotype is found on the C57 Black 6 background. This background is relatively more tumor resistant. Therefore, Osteopontin KO was first bred to the FVB background. Osteopontin KO on FVB background could then be bred to FGFR1

DNA From Tails

~.5 cm of tail from three week old mice was removed. Digestion buffer with Protease K was added to lyse the cells. Phenol chloroform was added to extract DNA.

Polymerase Chain Reaction

To detect Osteopontin mutant bands Osteopontin forward primers (5' – GTC TGG AGA ACA TGG GTG CT – 3') as well as Osteopontin KD primers (5' – GCC TGA AGA ACG AGA TCA GC – 3') were used. To detect Osteopontin wild type bands Osteopontin forward primers as well as Osteopontin WT primers (5' – GGG TGC AGG CTG TAA AGC TA – 3') were used. To detect FGFR1+ bands MMTV forward primers (5' – ACC TCT CGT GTG TTT GTG TCT – 3') as well as R1 reverse primers (5' CAT GGA TGC ACT GGA GTC AG – 3') were used. To compare Osteopontin mutant, Osteopontin wild type, and FGFR1+ bands PCR was run on a 1% agarose Biorad gel.

Whole Mounts

After fixing the glands in paraformaldehyde, acetone was added to defat the glands. They were then stained with a hematoxylin stain overnight (which can assist in explaining the darkness of the glands), followed by destaining and dehydration.

H & E Staining

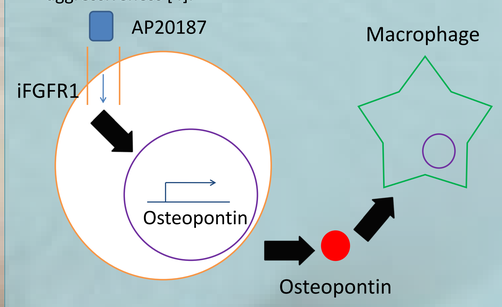
Paraffin sections were deparaffinized with Xylene then stained with a hematoxylin stain. They were then stained again with Eosin for contrast.

Background

Fibroblast growth receptors (FGFRs) and their ligands contribute to cellular functions including proliferation, survival, differentiation, migration, and angiogenesis [1]. When the FGFR1 chromosomal locus is amplified in breast cancer patients they do not respond well to current therapies and have been shown to develop resistance to endocrine therapies. To study FGFR1 signaling, an inducible FGFR1 (iFGFR1) system was engineered that can be activated by a synthetic dimerizer, AP20187.

Osteopontin

Osteopontin is a secreted glycophosphoprotein that is involved in a variety of different cancer types, including breast cancer. It is a pro-inflammatory protein that is upregulated by iFGFR1 activation. It is produced in a variety of tissues including the brain, liver, gastrointestinal tract, lung, bone, cardiac tissues, joints, and kidney [3]. It is also present in biological fluids including blood, urine, and milk, which makes it an attractive candidate for a novel breast cancer biomarker [4]. Higher levels of osteopontin mRNA or protein are found commonly in mammary tumor types as opposed to matching benign tissues. Data suggests that osteopontin is synthesized by breast carcinomas and acts to promote traits associated with increased aggressiveness [4].

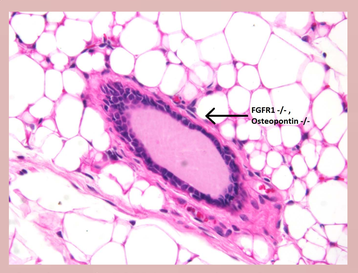


Dimerization of FGFR1 leading to activation of osteopontin transcription. Upon transcription and secretion, or osteopontin, migration of macrophages occurs.

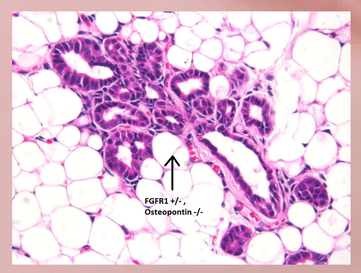
iFGFR1

iFGFR1 is expressed under the control of the mouse mammary tumor virus promoter (MMTV) so that FGFR1 can be specifically turned on in the mammary gland. iFGFR1 activation has been shown to promote increased angiogenesis, invasive lesions, and enhanced inflammatory response in our transgenic mice. It has also shown promotion of proliferation, survival, migration, and invasion of cells both *in vitro* and *in vivo* in nude mice [2]. Extended iFGFR1 activation in the mammary gland *in vivo* results in increased lateral budding in epithelial structures at the leading edges of mammary ducts, as well as development of hyperplasias, and multicellular invasive lesions which are characteristic of breast cancer progression [2]. All of these factors ultimately contribute to mammary tumor formation. Activation of iFGFR1 has been shown, by microarray analysis, to increase gene expression of osteopontin in mammary gland tissue from transgenic mice.

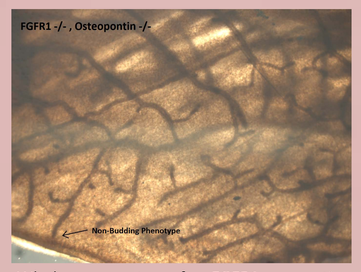
Results



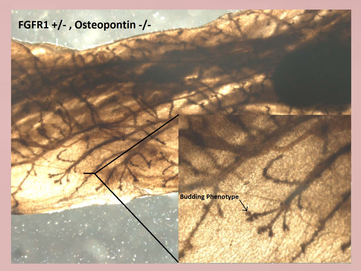
Section of FGFR1 negative, Osteopontin negative mammary gland with no budding phenotype.



Section of FGFR1 heterozygous, Osteopontin negative mammary gland with budding phenotype.

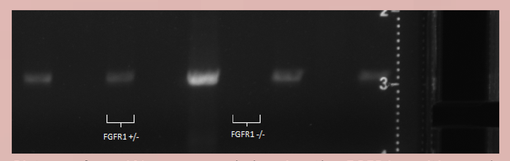


Whole mount of FGFR1 negative, Osteopontin negative mammary gland with no budding phenotype.



Whole mount of FGFR1 heterozygous, Osteopontin negative mammary gland with budding phenotype.

Picture of my 1% agarose gel showing different genotypes (Wild Type, Mutant, heterozygous)



Picture of my 1% agarose gel showing the FGFR1 positive and FGFR1 negative genotypes

Future Plans

Expression of osteopontin is required for macrophage recruitment *in vitro*. Blocking of osteopontin with an osteopontin-blocking antibody significantly reduces the number of macrophages that migrate, implying that secretion of osteopontin is necessary for migration of macrophages [4]. This recruitment of macrophages is also required for the lateral budding phenotype. Based on these observations, we hypothesize that iFGFR1-induced osteopontin plays a direct role in macrophage recruitment *in vivo*. Future studies will be conducted to observe osteopontin-macrophage interactions in mouse mammary glands *in vivo* using immunohistochemistry and other tissue staining methods with the F4/80 marker. Based on preliminary studies, we hypothesize that there will be a decrease in macrophage recruitment upon loss of osteopontin in the knocked out mouse model.

Acknowledgements

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References

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