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PVLSI (Pioneer Valley Life Sciences Institute) Posters - 2019

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Age-Dependent Alterations in Intestinal Wnt Signaling Revealed by Deletion of SFRP1

Briana Leung, Kelly Gregory, Jennifer Ser-Dolansky, Salih Toker, Sallie Schneider

PVLSI PIONEER VALLEY LIFE SCIENCES INSTITUTE — A Baystate Medical Center ■ UMass Amherst Research Partnership

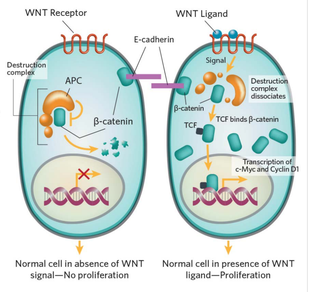
Baystate Health | University of Massachusetts Medical School — UMASS Medical School

BACKGROUND

Familial adenomatous polyposis disease (FAP) is a genetic disorder characterized by the early development of hundreds to thousands of adenomatous polyps throughout the colon. Left untreated, 100% of patients will inevitably develop colon cancer by age 40 and thus, surgical intervention with total abdominal colectomy or total proctocolectomy is ultimately required. The multi-stage progression from early adenoma to adenocarcinoma involves an initiating mutation in the APC gene, a negative regulator of the Wnt pathway.

Secreted frizzled related proteins (SFRPs) **are typically thought of as antagonists which** competitively binds to Wnt proteins, inhibiting the Wnt pathway and suppressing cancer. mRNA expression of SFRP1 is higher histologically normal mucosal areas relative to adjacent areas of colorectal carcinoma. rSFRP1 has been shown to induce apoptosis in human colorectal cells *in vitro* and suppress growth of human SQ colorectal cancer xenografts in mice. Aberration in the Wnt pathway is known to affect the proper development of stem cells (ISCs), Paneth and secretory goblet cells.

In this study we sought to confirm the notion of SFRP1 as an antagonist of the Wnt pathway in the small and large intestine, but interestingly found a pubertal role for SFRP1 in maintaining Wnt machinery expression, as well as AP4 and stem cell marker expression.



OBJECTIVE

- **To examine the role of SFRP1 in intestinal gene expression and development.**

METHODS

- SFRP1+/+ and SFRP1$^{-/-}$ mice were sacrificed at 5 weeks and 10-20 weeks of age (adults)
- After excision of intestinal tissue, the colon and small intestine (further divided into duodenum, jejunum and ileum) were flushed with ice-cold PBS to remove stool
- Tissues were fixed in formalin and embedded for H&E and PAS staining
- Tissue RNA was isolated and RT-PCR performed for Wnt pathway genes and *Ap4*

RESULTS

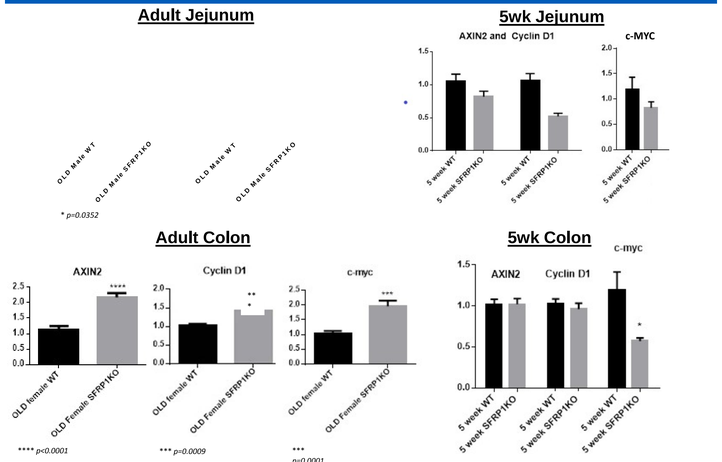


Figure 1. Gene expression of Wnt targets in 5wk old SFRP1$^{-/-}$ mouse intestine compared to adult SFRP1$^{-/-}$ mouse intestine

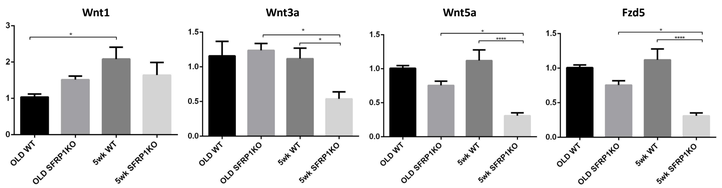


Figure 2. Gene expression of Wnt proteins and Fzd5 receptor in in 5wk old SFRP1$^{-/-}$ versus adult SFRP1$^{-/-}$ mouse intestine (jejunum)

RESULTS

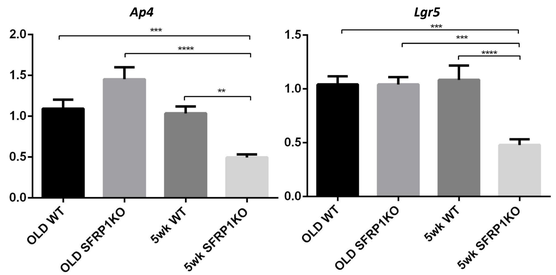


Figure 3. *Ap4* and *Lgr5* (stem cell marker) mRNA expression patterns in mouse jejunum

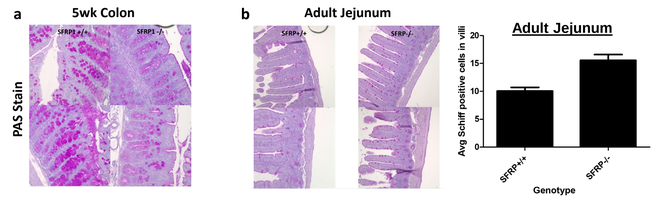


Figure 4. a PAS staining of colonic crypts in 5wk old WT vs. SFRP1$^{-/-}$ mice. **b** Left: PAS staining of small intestinal villi in adult mouse. Right: quantification of PAS-positive cells in jejunal villi adult mouse (20 villi)

CONCLUSIONS

- SFRP1 deficiency in adult animals resulted in increased target gene expression consistent with its role as an antagonist.
- Surprisingly, SFRP1 deficiency in pubertal mice was associated with a pattern of decreased Wnt pathway activation.
- The decrease in Wnt target gene expression in pubertal SFRP1 deficient animals was associated with decreased Wnt ligands and receptors suggesting an overall regulation of this receptor machinery.
- Ap4, a transcription factor known to regulate Wnt ligands/receptors, was significantly downregulated in the pubertal SFRP1-deficient animals suggesting a possible mechanism for the difference.
- An age-dependent change in Wnt activity uncovered by SFRP1 deficiency was confirmed by examining Wnt controlled development of intestinal goblet cells

Acknowledging Funding

Research presented in this poster was supported in part by the Natalie V. Zucker Research Grant from Tufts University School of Medicine

Developing organoid cultures for personalized medicine

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Abstract

There is great promise in personalized medicine for improving outcomes in cancer. However, genetic analyses are often slow and expensive and the combined effects of mutations on treatment responses may still be unknown. Thus, the development of new technologies that can test responses to a wide range of treatment is beneficial. Growth and treatment of cells in 2-dimensions have not yielded results that predict the in vivo responses. PDX animals, on the other hand, maintain the tumor heterogeneity and structure, but are time consuming and expensive. We are developing the technology of 3D organoid/spheroid culture. In this system the tumor is broken down into hundreds of small pieces and cultured in 3 dimensions in extracellular matrix allowing us to capture and propagate these genetic and phenotypic changes in the tumor organoids. Normal cell: cell interactions are noted and in the early passages of tumor pieces (spheroids) immune cells are also present allowing for examination of checkpoint blockade inhibitors.

In this work, we are showing growth of ovarian tumor and breast tumor, as well as normal tissue organoids/spheroids. We have developed immuno-histochemical and cytological techniques to analyze the cell types present or the activity of particular proteins. Future research aims to establish a panel of organoids to assess responses to novel treatments and to benefit other researchers interested in developing new treatment options.

A

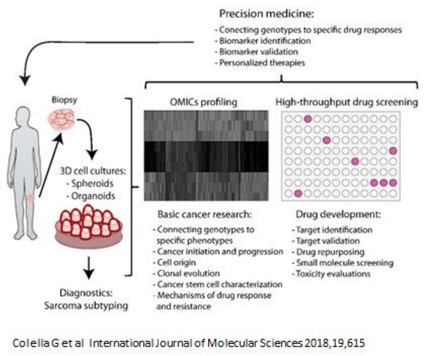

Figure 1.Schematic diagram showing potential use of organoids in personalized medicine. Hundreds of organoids or spheroids can be cultured and plated in a multi-well plate for evaluation of tumor responses. Genetic profiling can be coupled with it to study a wide range of biological questions.

Results

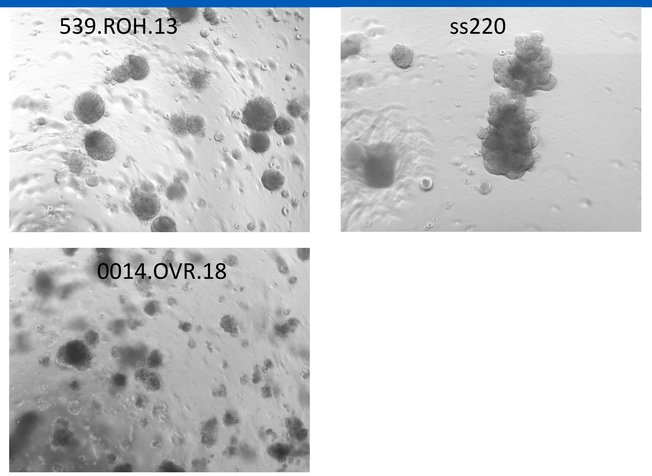

Figure 2. Organoid growth in 3D culture. A) Organoids derived from a breast tumor. B) Breast organoids derived from a reduction mammoplasty sample . C) Serous ovarian carcinoma organoids.

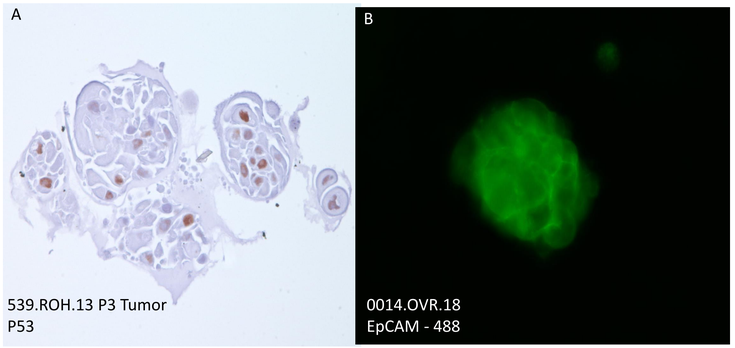

Figure 2: Immunohistochemistry / immunocytochemistry on breast and ovarian organoids A. Paraffin embedded breast tumor organoid stained with p53 antibody. B. Ovarian organoid in Matrigel stained fluorescently with EpCAM antibody.

Results

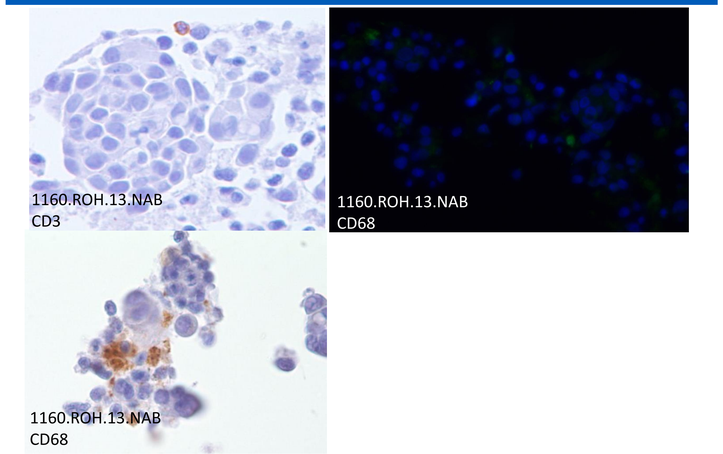

Figure 4. Immune cells can be detected in early passage spheroids Organoids were stained with antibodies to CD3 (T cells), CD45 (immune cells) and CD68 (macrophages).

Conclusions

- We are able to dissociate cells from both tumor and normal tissue and grow then in 3 dimension in extracellular matrix.
- We have been able to culture both breast and ovarian organoids.
- We have demonstrated several methods for staining cells which will allow us to demonstrate cellular heterogeneity, as well as changes in protein expression.
- Future studies will use these organoids to look at treatment responses or exposure risks.

Acknowledging Funding

Research was supported in part by the Rays of Hope Center for Breast Cancer Research and the IALS UMASS Amherst).

Development of a panel of human mammary epithelial cell lines for analysis of genetic variation on exposures

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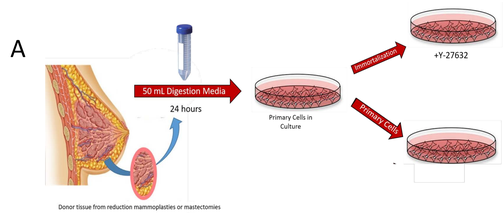
Abstract

Many studies that investigate toxicological and transformative changes of chemicals on the breast *in vitro* use the same 3-5 breast cell lines (i.e.MCF-7, MCF10A, MEC16, or 76N Tert cells). These lines have been useful in the study of various developmental and cancer pathways and have played an integral role in the development of treatment. What these lines are unable to provide is a diverse genetic background that allows for the study of interindividual variation between women. We have been able to isolate, and in some cases, conditionally immortalize more than 50 human mammary epithelial cell (HMEC) cell lines from different women enrolled in the Rays of Hope Breast Registry. We have been able to characterize these lines through a variety of different methods. We investigated the epithelial cell type, the mammosphere forming ability, as well as the differences in responsiveness to a variety of chemicals.

A



B

Cell Line	*KERATIN 14*	*KERATIN 5*	*VIMENTIN*	*KERATIN 18*
MCF10A	<1	<5	<1	<1
76N Tert	<1	<1	<1	<1
ME16C	<1	<5	<1	<1
HME-CC	<10	<10	<1	<1
554 P6	>10	>10	<1	<1
555 P16	>10	>10	<5	<1
563 P20	>10	>10	<1	<1
569 P8	>10	>10	<1	<1
698 P14	>10	>10	<1	<1
753 P12	<5	<5	<5	<1
811 P4	>10	>10	<1	<1
833 P4	<10	<10	<1	<1
847 P1	<1	<1	<1	<1
876 P6	>10	<5	<1	<1
1082 P4	<5	<10	<1	<1
1106 P2	<5	<5	<1	<1
1111 P5	>10	>10	<1	<1

Figure 1.Isolation and characterization of breast cells A. Fresh mammary tissue was minced and dissociated overnight mammary digestion media. The cell pellet was collected and further dissociated at which point cells were plated 24 hours in Mammocult media before being plated in T media specific for primary cell culture. At that point cells were then able to either be grown as described or plated for conditional immortalization on an irradiated NIH 3T3 murine feeder layer with the addition of ROCK inhibitor (Y-27632). **B.** Real-time qPCR characterization of established and primary HMEC lines to characterize between basal and luminal cell populations. *KERATIN14* and *KERATIN 5* are markers for the basal cell type; *VIMENTIN* is a marker for fibroblast-like cells; *KERATIN 18* is a marker for the luminal cell type.

Results

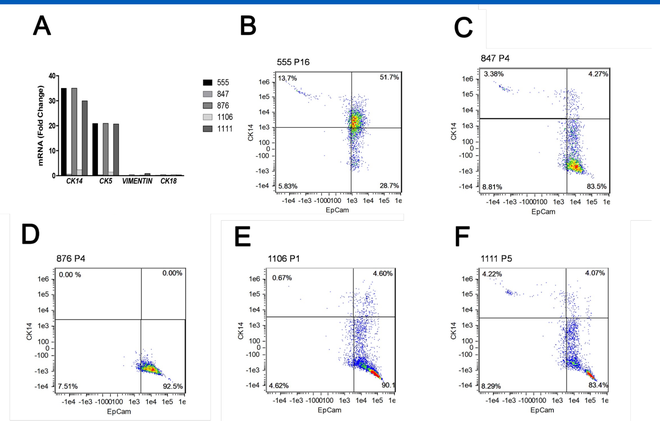


Figure 2. Flow cytometry to confirm the epithelial nature of the cells A. Representative real-time PCR data of cell lines used for fluorescent characterization. **B-F.** FACS analysis of several of the primary cell lines. Cells were stained with an EpCam specific antibody and a CK14 specific antibody. EpCam was used as a marker for epithelial cells and CK14 was used to confirm the basal cell type.

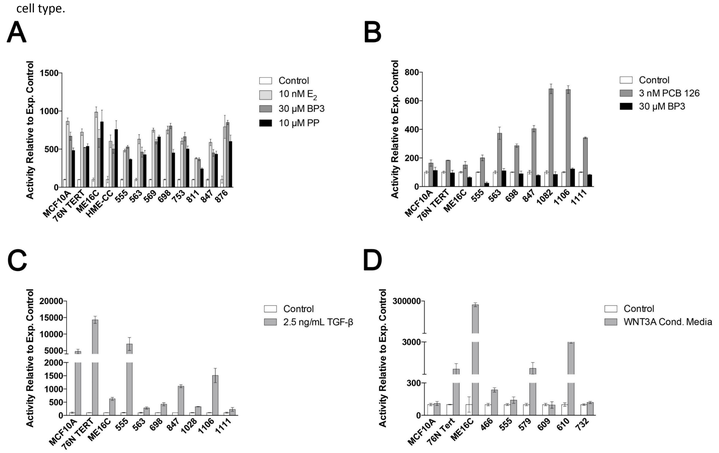


Figure 3. The panel displays variation in responses to chemicals, hormones and growth factors. Luciferase reporter assays were used to determine variation in response to various chemicals. Luciferase activity is shown normalized to Renilla transfection control. Activity is shown relative to experimental controls for each cell line. **A.** Stably and conditionally immortalized HMEC lines transfected with ESR1 expression plasmid and treated with control, 10nM E2, 30uM BP3, or 10uM PP media for 24 hours. ERE-luc luciferase activity was measured to observe variation in estrogen responsiveness. **B.** HMEC lines transfected with XRE-luc to measure differences in aryl hydrocarbon receptor in response to PCB 126. **C.** HMEC lines transfected with Caga-luc to measure differences in TGF-β when cells are treated with the TGF-β ligand. **D.** HMEC lines transfected with Topflash and treated with WNT3A conditioned media.

Results

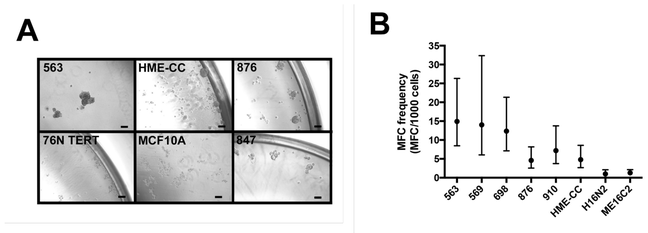


Figure 4. The conditionally reprogrammed cells retain the ability to form mammospheres Cells were cultured in ultra-low attachment 96-well plates for 7 days. **A.** Representative images of mammosphere formation in three HMEC lines (563, HME-CC, and 876) and examples of no mammosphere formation in three HMEC lines (76N TERT, MCF10A, 847). **B.** Extreme limiting dilution assays (ELDA) were performed to determine the number of MFC in each HMEC line. Only lines that had mammosphere in preliminary mammosphere forming assay were tested. Error bars represent 95% confidence interval. Scale bars indicate 100uM.

Conclusions

- We have established a panel of HMEC lines from women with associated demographic information to study interindividual variation.
- Examination of potential epithelial subtypes in our cultures demonstrate that our culture method enriches for basal-like epithelial cells.
- Some of our conditionally immortalized HMEC lines have mammosphere forming abilities in ultra-low attachment cultures.
- These lines can be used to examine the contribution of genetic variation to differential responsiveness to a variety of chemicals (i.e. PCBs or endocrine disrupting chemicals) or growth factors (i.e. Wnt or TGF-β).

Acknowledging Funding

Research was supported in part by the Rays of Hope Center for Breast Cancer Research and National Institute of Environmental Health Sciences of the National Institutes of Health U01ES026140 (DJJ, SSS)..

We would like to thank Ms. Eva Browne and Amy Roberts and for their help on these experiments

The Art of Physical Examination: Alcohol Abuse Masking Progressive Supranuclear Palsy

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University of Massachusetts Medical School-Baystate

BACKGROUND

Progressive supranuclear palsy (PSP) is an underdiagnosed neurodegenerative disease typically affecting adults in their middle and late ages. Those affected usually have a poor prognosis with an average life expectancy of 5.9 years. Physical examination is paramount for accurate diagnosis. Our case discusses PSP and the clinical features that may be masked by concomitant history of chronic alcohol abuse.

CASE PRESENTATION

RB is a 78-year-old male with a history of alcohol abuse, previous brain abscess status post craniotomy, traumatic subarachnoid and subdural hemorrhages, who was transferred from an outside hospital after multiple episodes of repeated falls at home and altered mental status with restlessness and agitation.

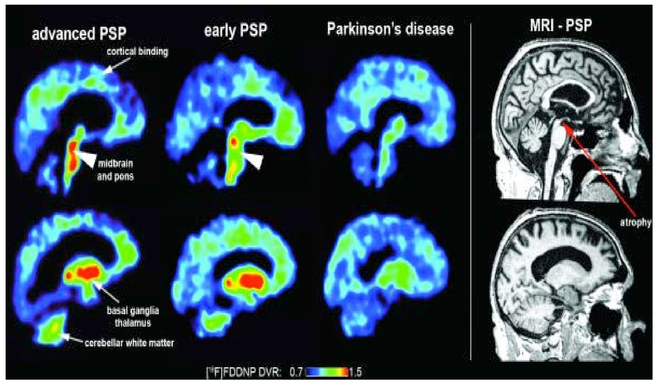
On examination, RB was found to be confused along with bilateral upper extremity resting tremors, which was noted on previous admissions as well. He had vertical gaze palsy and hypertonia in the upper limbs greater than the lower limbs. He also had cogwheel rigidity, masked facies, and a shuffling, bradykinetic gait.

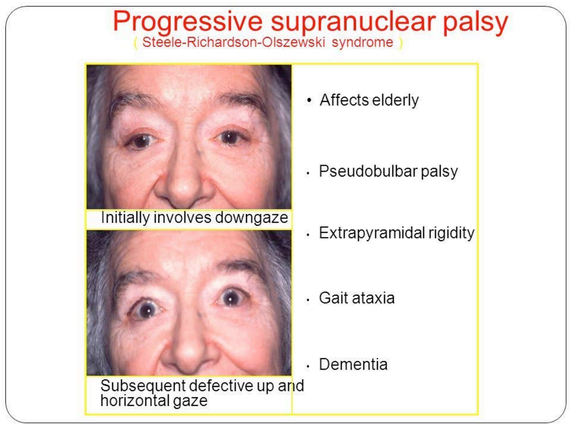
Lab work revealed hyponatremia of 130, a negative urine toxicology screen and undetectable levels of alcohol. Clinical Institute Withdrawal Assessment (CIWA) was 12 on admission. CT brain ruled out any underlying acute abnormalities.

The patient had been admitted in the past for acute pancreatitis, repeated falls and brain injuries secondary to alcohol intoxication. Given his known history of alcohol abuse, he was treated for alcohol withdrawal with lorazepam without any improvement in mentation.

ANOTHER HEADING

Geriatrics was consulted once other infectious and metabolic causes were ruled out. A thorough physical exam revealed that patient had vertical downward gaze with history of “backward” falls. Follow up MRI showed marked atrophy of midbrain tegmentum which confirmed PSP.





DISCUSSION

This case highlights how a history of alcohol abuse may delay the diagnosis of neurodegenerative diseases such as PSP due to overlapping features of cognitive impairment, eye movement disorders and cerebellar ataxia. However downward gaze palsy on examination and brain imaging findings can differentiate PSP from alcohol abuse. Unfortunately, there is no treatment of PSP as dopaminergic medications show only transient or limited relief in symptoms.

CONCLUSION

A lack of response to levodopa makes PSP a more likely diagnosis than Parkinson’s disease. PSP is usually diagnosed clinically, but can be supported by magnetic resonance imaging. This may demonstrate severe pigment depletion of the substantia nigra. Unfortunately, there is no treatment of PSP as dopaminergic replacement therapy is only transiently or mildly effective in relieving some symptoms.

Acknowledging Funding

•Progressive supranuclear palsy. *Postgrad Med J*. 2000;76(896):333-6.

The use of patient-derived breast tissue explants to study resident macrophage polarization and the effects of xenoestrogen exposure

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ABSTRACT

Using immortalized human cell line models *in vitro* or *in vivo* has aided researchers in the understanding of how normal mammary epithelial cells respond to neighboring cell signals. Our laboratory is in charge of ongoing collections of breast tissue for the Biospecimen Resource and Molecular Analysis facility at Baystate Medical Center. The continuous access to fresh breast tissue has allowed us to employ an *ex vivo* mammary explant model system comprised of intact human mammary tissue which is termed patient derived explant (PDE). This glandular tissue contains all the cells that would normally be present in the breast. As such, our collection of PDEs allows us to investigate cellular responses to external stimuli *in situ* in cells that are all normal and have normal heterotypic interactions. Macrophages comprise a portion of immune cells that are phagocytic in nature and are present in almost all tissues. In the breast, macrophages play an important role in ductal/lobular development. Depending on the microenvironmental signal present, macrophages are polarized into two distinct phenotypes, classically activated M1 macrophages (proinflammatory) or alternatively activated M2 macrophages (wound healing and/or anti-inflammatory). Here we demonstrate that that we can polarize tissue resident macrophages within normal breast PDEs towards M1 or M2 through the addition of IFNγ + LPS or IL-4 + IL-13 respectively. Elevated expression levels of M1 markers (HLA-DRA and CXCL10) or M2a markers (CD209 and CCL18) are observed in cytokine treated tissues. Our *ex vivo* culture system further reveals that a subset of the PDEs respond to M2 polarizing cytokines through down regulation of E-cadherin and upregulation of Vimentin which is reminiscent of EMT changes observed in cancer cells or “active” stromal phenotypes. Furthermore, these changes highlight a variation in inter-individual responses that could possibly contribute to differences in susceptibility to breast cancer. We have continued these PDE studies and used this model system to examine the impact of environmental exposures on the mammary gland. In particular, we are focusing on a xenoestrogen, oxybenzone (BP3), which is the active ingredient found in sunscreen. Considering that estrogen has been shown to increase the production of M2 related cytokines, we sought to determine whether BP3 could affect macrophage polarization in PDEs. We measured significant, yet varying responses to BP3 particularly in untreated and M2-cytokine treated PDEs. However, it is of interest that BP3 had no effect on EMT related gene expression. Taken together, the PDE model system is an outstanding preclinical model to study early tissue resident immune responses and effects on epithelial and stromal responses to stimuli found both endogenously in the breast as well as exogenously due to exposures.

METHODS

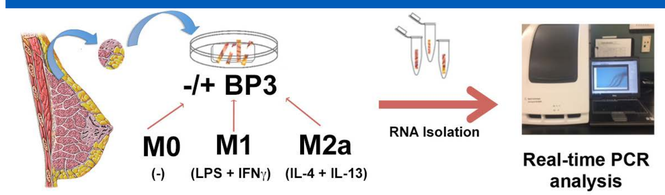

Figure 1. Schematic for human tissue explant exposures. Fresh human breast tissue dissected to isolate fibroglandular tissue enriched with epithelium was placed on Surgifoam gelatin sponges with 3 mL of medium [(phenol red free DMEM/F12 buffered with Hepes and $NaHCO_3$, 5 µg/mL human insulin, 1X antibiotic/antimycotic. The media was left untreated (M0) , treated with M1 polarizing cytokines (LPS + IFNγ) or treated with M2a polarizing cytokines (IL-4 + IL-13). Additionally, in parallel wells the media was supplemented with benzophenone-3 (BP3). Media was removed after 72 hr and the tissue was flash frozen and stored at -80°C until being processed for RNA isolation.

RESULTS

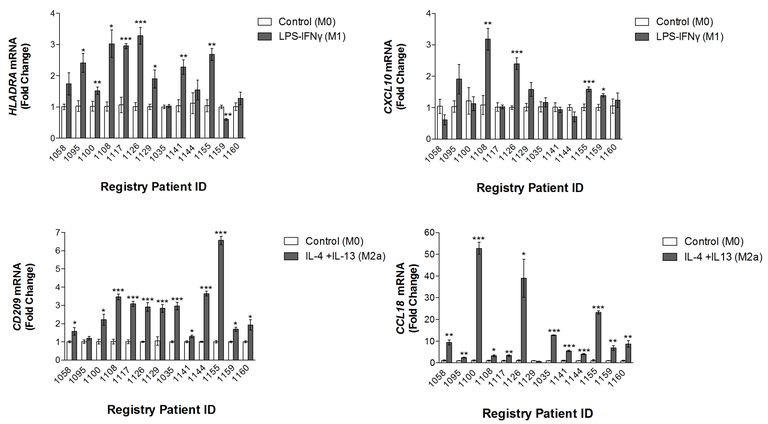

Figure 2. Normal breast PDEs can be polarized towards M1 or M2 through the addition of IFNγ + LPS or IL-4 + IL-13. RNA was harvested from cytokine exposed PDEs and mRNA levels of *HLA-DRA* and *CXL10* (M1 markers) or *CD209* and *CCL18* (M2a markers) were analyzed via real-time PCR. All real-time PCR results are from two separate experiments performed in triplicate and results were normalized to amplification of *CD68* (macrophage marker). Bars represent mean ± SEM and are expressed as fold change with respect to M0 PDEs. *p<0.05, **p<0.01, ***p<0.001 (significantly different from indicated data set using student’s *t*-test).

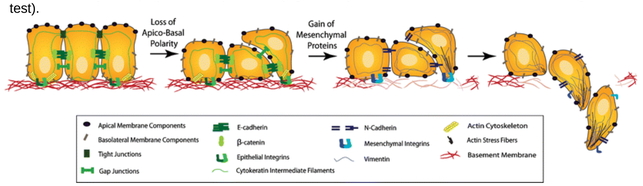

Figure 3. Schematic of epithelial to mesenchymal transition (EMT). EMT is a process whereby epithelial cells lose polarity as well as cell-cell contacts and undergo a dramatic remodeling of the cytoskeleton resulting in a mesenchymal morphology. Image obtained from https://upload.wikimedia.org/wikipedia/commons/8/89/Epithelial-Mesenchymal_Transition.gif

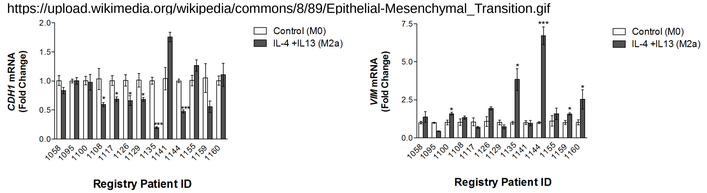

Figure 4. Epithelial cells in PDEs exhibit a gene expression pattern associated with EMT when macrophages are polarized toward the M2a phenotype. RNA was harvested from IL-4 + IL-13 exposed PDEs and mRNA levels of E-cadherin *(CDH1)* and Vimentin *(VIM)* were analyzed via real-time PCR. All real-time PCR results are from two separate experiments performed in triplicate and results were normalized to amplification of CK 18 (epithelial cell marker). Bars represent mean ± SEM and are expressed as fold change with respect to M0 PDEs. *p<0.05, ***p<0.001 (significantly different from indicated data set using student’s *t*-test).

RESULTS

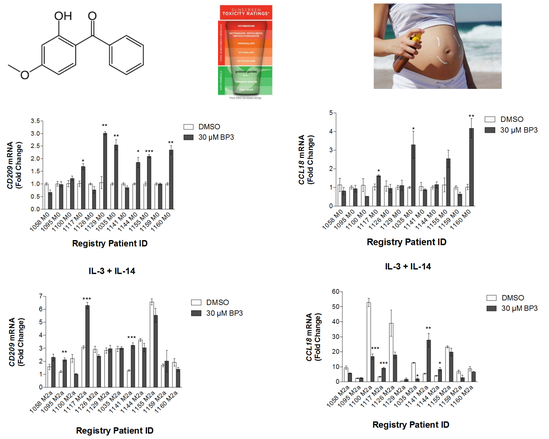

Figure 5. The xenoestrogen oxybenzone (BP3) alters the expression of M2a markers in both untreated PDEs and those treated with IL-4 +IL-13. RNA was harvested from control (M0) and cytokine exposed (M2a) PDEs treated with either vehicle (DMSO) or 30 µM BP3 and mRNA levels of *CD209* and *CCL18* (M2a markers) were analyzed via real-time PCR. All real-time PCR results are from two separate experiments performed in triplicate and results were normalized to amplification of *CD68* (macrophage marker). Bars represent mean ± SEM and are expressed as fold change with respect to DMSO treated M0 PDEs. *p<0.05, **p<0.01, ***p<0.001 (significantly different from indicated data set using student’s *t*-test).

CONCLUSIONS

- The resident macrophages found in normal breast tissue PDEs are capable of responding to polarizing cytokines as evidenced by the expression of classic M1 and M2a markers and inter-individual variation is noted in the responses.
- In a subset of women, M2a polarized macrophages alter gene expression consistent with EMT process. This is the first study to show the effects of M2a macrophage polarization on EMT in NORMAL epithelial tissue.
- BP3 does not affect M1 macrophage polarization or EMT gene expression, but in some cases does elicit significant changes in M2a macrophage marker gene expression which could potentially alter breast cancer susceptibility.

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