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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

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 surprisingly 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

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

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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.

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.

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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 them 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).

UMMS-Baystate Research & Education: Together we advance the state of caring through discovery & innovation
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.

Abstract

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Results

Figure 1. Isolation and characterization of breast cells. A. Fresh mammary tissue was minced and dissociated overnight inMammary digestion media. The cell pellet was collected and further dissociated at which point cells were plated 24 hours in Ultra-low attachment 96-well plates for 7 days. B. Representative images of mammosphere formation in three HMEC lines (563, HMECC, and 569) and examples of no mammosphere formation in three HMEC lines (TEN TERT, MCF10A, MCF1A). C. Extreme limiting dilution assay (ELDA) were performed to determine the number of MFC in each HMEC line. Only lines that had mammospheres in a preliminary mammosphere forming assay were tested. Error bars represent 95% confidence interval. Scale bars indicate 500 μm.

Figure 2. Flow cytometry to confirm the epithelial nature of the cells. A. Representative real-time PCR data of cell lines used for in vitro testing. B. H&E images of primary mammary cells. 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 epithelial cells.

Figure 3. The panel display variation in response to chemicals, hormones and growth factors. Luciferase reporter assays were used to determine variation in response to various chemicals. Luciferase activity is shown normalized by transfection control. Activity is shown relative to experimental controls for each cell line. A. Luciferase activity associated with 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).

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, HMECC, and 476) and examples of no mammosphere formation in three HMEC lines (TEN TERT, MCF10A, MCF1A). B. Extreme limiting dilution assay (ELDA) were performed to determine the number of MFC in each HMEC line. Only lines that had mammospheres in a preliminary mammosphere forming assay were tested. Error bars represent 95% confidence interval. Scale bars indicate 500 μm.

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).

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UMMS-Baystate Research & Education: Together we advance the state of caring through discovery & innovation
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.

BACKGROUND

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.

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.

DISCUSSION

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.

CONCLUSION

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 vascular 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 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 we can polarize tissue resident macrophages within normal breast PDEs towards M1 or M2 through the addition of IFNγ + LPS or L-4-4 + γ-13 respectively. Elevated expression levels of M1 markers (HLA-DRA and CCL11) or M2a markers (CD209 and CCL18) were 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 responses to stimuli found both endogenously in the breast as well as exogenously due to exposures.

METHODS

RESULTS

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, intact 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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