Introduction

Medullary Thyroid Carcinoma (MTC) is a relatively rare cancer that occurs both sporadically and in the setting of inherited RET protooncogene mutations associated with Multiple Endocrine Neoplasia Type 2. Our goal was to review our local experience with MTC.

Methods & Materials

A retrospective study was performed of all patients undergoing thyroidectomy for a diagnosis of thyroid cancer in a single institution from 01/01/2001-12/31/2011. Charts were reviewed for demographic variables, operative notes, pathology reports, and biochemical data.

Results

A total of 352 patients were identified who underwent thyroidectomy for thyroid cancer. Of these, 14 (4%) patients had a diagnosis of MTC. All but one patient underwent initial thyroidectomy at our high-volume thyroid center; that patient underwent thyroid lobectomy in the community for a thyroid nodule and was referred for further management once the diagnosis of MTC was made. One patient underwent thyroid lobectomy at our center for unilateral goiter; when MTC was diagnosed on final pathology, the patient did not follow up for additional surgical management. One patient underwent prophylactic thyroidectomy for known MEN2A; the remainder of the patients underwent therapeutic thyroidectomy and appropriate lymph node dissection. Results of genetic testing for MEN2 were available for 5 patients; 4 tested positive for MEN2A and 1 tested negative.

Conclusion

Although our experience with MTC is small, we found that MTC represents approximately 5% of all thyroid cancers treated at our center. This is in line with the incidence rates seen in the literature. It was difficult through retrospective chart review to determine decision-making algorithms and outcomes. This may be specific to our region, where referrals and care tend to be fragmented. A protocol-based multidisciplinary approach for these patients would ensure that patients receive appropriate treatment and follow-up.

References


Acknowledgements

Many thanks to the Division of Surgical Oncology, University of Louisville Department of Surgery and NCI R25 grant support University of Louisville Cancer Education Program NIH/NCI (R25-CA134283) for making this opportunity possible.
ASSESSING THE HARVARD SCALE OF BREAST COSMESIS: AN ANALYSIS OF PATIENTS TREATED WITH BREAST CONSERVING THERAPY ON A PHASE II CLINICAL TRIAL

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Department of Radiation Oncology, James Graham Brown Cancer Center; University of Louisville School of Medicine, Louisville, Kentucky

ABSTRACT

Purpose: Cosmetic outcome is frequently used to assess quality of life (QOL) outcomes in patients that have undergone treatment for breast cancer. As such, an evaluation of the quality and objectivity of the Harvard Scale, a commonly-used assessment tool utilized to evaluate cosmetic outcome in breast cancer patients, is important in gauging objective and congruent QOL expectations following breast cancer treatment.

Materials and Methods: Seventeen (17) patients enrolled on a Phase II protocol, involving Accelerated Hypofractionated Radiotherapy after Breast Conserving Surgery for early stage breast cancer treatment, were assessed at four post-operative time points within a one-year window following radiation treatment. Each time point assessment was completed by the treating physician using the four-tier Harvard Scale (Excellent, Good, Fair, Poor) to compare the treated breast to the controlled breast. A survey including the same de-identified images was then distributed to forty-four (44) medical students and residents in hopes of assessing the validity of the Harvard Scale.

Results: Of the 44 medical student and resident respondents, there was equal distribution between male (23) and female (21), with the majority of respondents describing themselves as single (82%), Caucasian (70%), and less than twenty-nine (29) years of age (89%). The physician’s assessment of cosmetic outcome differed somewhat from the respondent averages: Excellent (43% vs. 27%), Good (34% vs. 34%), Fair (20% vs. 28%) and Poor (3% vs. 11%). Congruence between the physician and the respondents in the same four categories showed to be 49%, 37%, 44%, and 86%, respectively. Finally, when combining the categories into a bimodal representation of Excellent/Good (70%) versus Fair/Poor (72%), there was a significant increase in congruence between the treating physician and the respondents.

Conclusion: Evaluation of cosmetic outcome following treatment for early stage breast cancer can be a subjective process, often differing from evaluator to evaluator. Our results suggest that a two point scale in place of the four-point Harvard Scale would be a better method to evaluate cosmetic outcome due to the significant improvement in congruity among evaluators.

DATA ANALYSIS GRAPHS

THE HARVARD SCALE OF BREAST COSMESIS

Please assess breast cosmesis at this time. (Circle the number next to the word that best describes the cosmetic results.)

1. EXCELLENT: when compared to the untreated breast or the original appearance of the breast, there is minimal or no difference in the size or shape of the treated breast. The way the breast feels (its texture) is the same or slightly different. There may be thickening, scar tissue or fluid accumulation in the breast, but not enough to change the appearance.

2. GOOD: there is a slight difference in the size or shape of the treated breast as compared to the opposite breast or the original appearance of the treated breast. There may be some mild reddening or darkening of the breast. The thickening or scar tissue within the breast causes only a mild change in the shape or size.

3. FAIR: Obvious differences in the size and shape of the treated breast. This change a quarter or less of the breast. There can be moderate thickening or scar tissue of the skin and the breast, and there may be obvious color changes.

4. POOR: marked change in the appearance of the treated breast involving more than a quarter of the breast tissue. The skin changes may be obvious and detract from the appearance of the breast. Severe scarring and thickening of the breast, which clearly alters the appearance of the breast, may be found.

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NIH/NCI R25 grant support
Identifying urine exosome miRNA profiles in melanoma patients

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Departments of Surgery1, University of Louisville School of Medicine 
Microarray Facility2, University of Louisville School of Medicine

Abstract

Background: Melanoma is a highly heterogeneous malignant tumor with a wide range of progression among stages based on the American Joint Committee on Cancer (AJCC) groupings. A need exists for developing biomarkers that can aid in melanoma diagnosis, prognosis, and follow-up. Exosomes are 40-100nm diameter membrane vesicles released from tumors into the body fluids such as urine. Unique miRNA in tumor exosomes contributes to malignant progression through post-transcriptional silencing of mRNA. This leads to the possibility of characterizing differences in expression of exosomal miRNAs of melanomas and non-melanoma patients in the search for a biomarker for assessment of the disease process.

Methods: This study used urine collected from non-melanoma, Stage I, and Stage IV melanoma patients to isolate exosomes. Western blot and electron microscopy were used to assess purity of exosomes. Urine exosome RNA was isolated and applied to Affymetrix miRNA array 2.0. Differentially expressed miRNAs were found in Stage I vs Control group. Among them were miR-22, miR-181, miR-191, miR-192, miR-199a, miR-93, and miR-100. In comparison of Stage IV vs Stage I melanomas patients, there were 5 miRNAs (miR-132, miR-197, miR-198, miR-30b, and miR-494) with significant changes. There were 9 significant expressed miRNAs detected in both Stage I vs Control and Stage IV vs Control groups. A database search of these miRNAs function revealed some of the miRNAs have been previously implicated in cancer process, but many have no known function.

Conclusions: To our knowledge, this is the first attempt to analyze urine exosomal RNA profiles in melanoma patients. These distinctive urine exosomal miRNA signatures may be used as diagnostic and prognostic markers for melanoma patients. This research could lead to translation into a non-invasive method for identifying diagnostic and prognostic biomarkers in melanoma patients.

Introduction

• Exosomes are 40-100nm membrane bound vesicles excreted in excess from tumor cells into body fluids such as urine.
• Exosomal microRNA (miRNA) have recently been shown to play a role in malignant progression.
• miRNA is small, noncoding RNA that posttranscriptionally silences mRNA.
• Analyzing exosomal miRNA signatures in melanoma patients could identify a potential noninvasive biomarker.

Methods

Patient Groups:
• Non-melanoma
• Stage I Melanoma
• Stage IV Melanoma

Exosomes were isolated from urine using a modified ExoQuick-TC protocol. Western blot and electron microscopy were used to assess purity of exosomes.

RNA was isolated from exosome and miRNA array was conducted using Affymetrix miRNA array 2.0.

Differentially expressed miRNAs across the three sample groups were analyzed by ANOVA.

Results

Table 1. Differential expressed miRNAs (stage I vs. non-melanoma) (30.80, >2FC>2) (4 shown of 33 found)

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Stage I</th>
<th>Non-melanoma</th>
<th>Stage IV</th>
<th>p-value</th>
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Table 2. Differential expressed miRNAs (stage I vs. non-melanoma) (30.80, >2FC>2) (4 shown of 33 found)

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Table 3. Differential expressed miRNAs (stage IV vs. non-melanoma) (30.80, >2FC>2) (4 shown of 17 found)

<table>
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Table 4. Differential expressed miRNAs (stage IV vs. stage I) (p<0.05, >2FC>2) (2 shown of 5 found)

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Table 5. Overlapped differential expressed miRNAs (stage I vs. non-melanoma and stage IV vs. non-melanoma) (p<0.05, >2FC>2) (5 shown of 9 found)

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Conclusions

• Larger amounts of urine exosomes were found in melanoma patients than those in non-tumor patients.
• There are specific exosomal miRNAs found in Stage I and IV melanoma patients compared to non-melanoma patients.
• While some of these specific miRNAs have been previously associated with malignant tumors, most have no known function and could be specific melanoma markers.
• These results provide a starting point for identifying a miRNA signature of melanoma-derived exosomes. The clinical translation into a non-invasive diagnostic and prognostic biomarker is a real possibility.

Acknowledgements

Research supported by NCI R25 grant University of Louisville Cancer Education Program NRHNCI (R25-CA134283), grant from Melanoma Research Foundation, and University of Louisville Clinical & Translational Science Pilot Grant Innovate Award to K.M.M.
Translocase Activity in Parotid Acinar Cells

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Introduction

Salivary glands have a critical role in maintaining the dynamic equilibrium of the oral cavity. Saliva has antimicrobial properties, lubricates food for swallowing and initiates carbohydrate digestion. Salivary gland cancers occur in approximately one in every 50,000 people each year in the United States. Recent studies suggest that translocases (adenine nucleotide translocase) or flippases/floppases (P-glycoprotein⁴, MDR1², ABCB1¹) are factors in eliciting cancer cell death and promoting therapeutic targets. Additionally, lipid transporters such as ATP11A, ABCG1 or PLSCR2 may play a role in sorting of salivary secretory proteins in the parotid gland. Our objective was to identify which translocases or flippases/floppases are present in the parotid gland and determine their location in the acinar cell.

Methods

The presence of translocases or flippases/floppases was determined by PCR and immunocytochemistry. Total RNA was isolated from the parotid glands of healthy adult Sprague-Dawley rats and quantitated. cDNA from the total RNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) following the manufacturer’s protocol. The cDNA was then used for analytical and quantitative PCR. Primers for specific flippases/floppases or translocases were designed from mRNA sequences selected from NCBI nucleotide databases. The analytical PCR products were visualized by agarose gel (1%) electrophoresis. The mRNA levels for the selected proteins were quantitated by real-time PCR using the Quantitect SYBR Green kit protocol and the expression normalized to GAPDH. A few select proteins were localized by immunofluorescence on paraffin-embedded adult parotid tissue sections. Prior to immunohistochemistry (IHC), the parotid sections were subjected to antigen retrieval. The tissue was then blocked with 10% normal goat serum in phosphate buffer saline, pH 7.3 (PBS) and incubated overnight with primary antibodies either to ABCA1 or ABCG1. Following washes with PBS, the sections were incubated with FITC-tagged anti-rabbit IgG, washed and the binding visualized on an Olympus FV500 confocal microscope.

Background

Parotid secretory protein (PSP) is an excellent marker for regulated secretion from the parotid gland. Prior work in the laboratory has demonstrated that PSP binds to phospholipid bilayer-3,4-phosphate (PI3,4P₂) in the secretory granule membrane and thus be potentially sorted into the regulated secretion pathway. The presence of reverse scramblases (CABC1 and DP1AP3) from the granule outer membrane leaflet where it is synthesized to the inner leaflet wherein it binds to the cargo protein.

Results

- The presence of translocases or flippases/floppases was determined by PCR and immunocytochemistry. Total RNA was isolated from the parotid glands of healthy adult Sprague-Dawley rats and quantitated. cDNA from the total RNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) following the manufacturer’s protocol. The cDNA was then used for analytical and quantitative PCR. Primers for specific flippases/floppases or translocases were designed from mRNA sequences selected from NCBI nucleotide databases. The analytical PCR products were visualized by agarose gel (1%) electrophoresis. The mRNA levels for the selected proteins were quantitated by real-time PCR using the Quantitect SYBR Green kit protocol and the expression normalized to GAPDH. A few select proteins were localized by immunofluorescence on paraffin-embedded adult parotid tissue sections. Prior to immunohistochemistry (IHC), the parotid sections were subjected to antigen retrieval. The tissue was then blocked with 10% normal goat serum in phosphate buffer saline, pH 7.3 (PBS) and incubated overnight with primary antibodies either to ABCA1 or ABCG1. Following washes with PBS, the sections were incubated with FITC-tagged anti-rabbit IgG, washed and the binding visualized on an Olympus FV500 confocal microscope.

Discussion

It is important to understand acinar cell differentiation and transport mechanisms to reactivity or regenerate the cell systems and tissue, respectively, in patients with loss of salivary gland function. IHC revealed localization of the cell membrane lipid translocation protein ABCA1 near apical and basolateral parotid acinar cell membranes and potentially secretory vesicles. ABCA1 could be involved in parotid acinar cholesterol transport since its primary mechanism throughout the body is cholesterol transport. ABCG1 did not localize. ATP11A and CABC1 had the highest mRNA expression and should be examined under IHC.

Conclusions

Several flippases/floppases or scramblases that could be potentially involved in the secretory process are found in the healthy parotid gland. ATP11A and CABC1 had the highest levels of mRNA expression of those studied. IHC revealed localization of ABCA1 in the cell membrane and possibly in secretory granule membranes. Further analysis of these proteins will help elicit their role in protein sorting in the parotid gland.

Acknowledgements

Research supported by a grant from the National Cancer Institute grant R03-CA14423.

References


CaMKII represses CaMKIV transcription to promote proliferation in myeloid leukemia cells
Lauren Strait1, Cuibo Yang3, Maddalena Illario2 and Uma Sankar1,2,3,4
University of Louisville School of Medicine1; Owensboro Cancer Research Program2
and James Graham Brown Cancer Center3; Department of Pharmacology and Toxicology4, University of Louisville and
Dipartimento Biologia e Patologia Cellulare e Molecolare, Federico II University5

INTRODUCTION
Calcium/Calmodulin dependent protein kinases (CaMK) play an important role in cell signaling cascades by linking increases in intracellular calcium with biological processes. In myeloid leukemia cells, calmodulin dependent protein kinase II (CaMKII) promotes cell proliferation by binding to RAS/RAF and stabilizing the mitogen-activated protein kinase (MAPK) pathway. CaMKII also binds to and phosphorylates retinoic acid receptor (RAR), a nuclear hormone receptor transcription factor. This phosphorylation of RAR increases its interaction with co-repressors causing an inhibition of RAR-mediated gene transcription. Alternatively, CaMKIV is expressed at very low levels in myeloid leukemia cells. However, pharmacological inhibition of CaMKII enhances CaMKIV mRNA and protein expression, potentially through the de-repression of CaMK4 transcription. The CaMK4 promoter contains RAR response element (RRE) that can be activated by retinoic acid. Since CaMKII can inhibit RAR-mediated transcription, we hypothesize that increased CaMKII can repress CaMKIV transcription. In this study, we investigated the potential cross talk between CaMKII and CaMKIV in multiple myeloid leukemia cell lines.

MECHANISM

RESULTS

CONCLUSION
Repression of CaMK4 transactivation by CaMKII has previously been proven in our lab using HEK 293 cells as the transfected target cell. The purpose of this study was to investigate whether this occurs in vivo, in leukemia cells. The U937 and K562 cell lines cannot be easily transfected, so a ChIP assay was utilized instead. Using the ChIP assay we established the baseline for RAR-alpha binding on CaMKIV promoter in the myeloid leukemia cell lines. The next step in this process is to inhibit CaMKII using KN-93 and to see how this effects CaMKIV expression.

REFERENCE
Determining the relationship between ubiquilin 1 and insulin-like growth factor 1 receptor in HEK 293T cells.

Jara Vega Velez, Levi Beverly, PhD
University of Louisville, Louisville, KY

Background

Ubiquilin1 (UBQLN1) is a protein that binds to ubiquitinated proteins and either takes the protein to the proteasome for degradation or protects the protein from degradation. UBQLN1 has been shown to play a role in protection and degradation of different proteins that may play a role in cancer. Insulin-like growth factor 1 receptor (IGF1R) activity has been linked to cancer and we have shown that IGF1R interacts with UBQLN1. IGF1R is a tyrosine kinase receptor that is activated by insulin-like growth factor 1 and insulin-like growth factor 2. Upon stimulation, IGF1R is auto-phosphorylated and begins a signaling cascade that is vital for cell proliferation and survival.

Objective

The objectives of this experiment are:
• To confirm a relationship between UBQLN1 and IGF1R.
• To determine the effect of UBQLN1 on the persistence of phosphorylated IGF1R in cells.
• To compare the effects of UBQLN1 deletion mutants on the persistence of phosphorylated IGF1R in cells.

Results

Figure 1: Schematic of IGF1R signaling

Figure 2: Cells treated with complete DMEM media or PBS for 5 minutes.

Figure 3: Cells treated with complete DMEM media or PBS for 15 minutes.

Figure 4: Cells treated with complete DMEM media or PBS for 5 or 15 minutes.

Future endeavors

Future experiments:
• To determine if effect of UBQLN1 on the persistence of phosphorylated IGF1R in cells starved only of growth factors compared to cells starved of growth factors and nutrients.
• To determine when activation of IGF1R returns to cells after a period of starvation.
• To determine if UBQLN1 plays a role in the return of IGF1R activation after a period of starvation.

Acknowledgments

We thank NCI R25 grant support, University of Louisville Cancer Education Program NIH/NCI (R25-CA134283), and Kosair Pediatric Cancer Program for funding support.