

500 Graft-Versus-Host Disease Has Feature of T Cells, B Cells, and Systemic Sclerosis: A Study of Cytokine Expression in Histologically Matched Skin Biopsies

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Background: Graft-versus-host disease (GVHD) is the leading cause of non-relapse mortality after allogeneic bone marrow transplantation (BMT). Although the clinical presentation is well-characterized, much remains to be elucidated in the context of pathogenesis.

Design: Forty-seven skin biopsies representing GVHD grades 0, I, II, III, lichenoid (L), and sclerodermoid (S) were included from 31 patients with previous allogeneic BMT. Paraffin embedded tissue was harvested for cDNA. Real-time polymerase chain reaction assessed levels of the following markers: 1) T and B cells: CD3, CD20; 2) T cell subsets: Foxp3, TGF β , IL-17; 3) cytokines associated with GVHD: interferon-gamma (IFN γ), IL-6; 4) factors implicated in systemic sclerosis: connective tissue growth factor (CTGF), allograft inflammatory factor-1 (AIF-1), and IL-13. Expression was correlated to grade, length of survival, and overall survival. Statistical analysis was performed using logistic and linear regressions.

Results: Levels of two markers significantly correlated with length of survival (TGF β , corr coeff -23.6, $p=0.009$ and AIF-1, 14.0, $p=0.035$), while IFN γ approached statistical significance (-11.2, $p=0.076$). Levels of the same three markers also correlated with histologic grade by inspection, but did not reach statistical significance: IFN γ (0, 0.251, I, 0.599, II, 0.767, III, 0.496, L, 1.02, S, 0.382, $p=0.097$), TGF β (0, 0.396, I, 0.544, II, 0.876, III, 0.570, L, 0.926, S, 173.6, $p=0.13$), and AIF-1 (0, 0.124, III, 0.006, L, 0.728, S, 0.718, $p=0.527$). No correlation to diagnosis or survival was found with CD3, Foxp3, IL-6, and CTGF. Expression of IL-17, IL-13, and CTGF was not detected. Lastly, expression of CD20 was observed in 2 of 9 patients with lichenoid GVHD.

Conclusions: We identify 3 markers that are not only potentially implicated in the progression from acute to chronic GVHD, but also modulate survival after transplantation, rendering IFN γ , AIF-1, and TGF β promising therapeutic targets. A second target is identified in a subset of patients with lichenoid GVHD. The presence of CD20 positive B cells may correlate with the recently described group of chronic GVHD patients who are responsive to rituximab. Our findings challenge the notion that GVHD is a uniform, T cell driven process. Rather, there appears to be subsets within GVHD patients, whose disease manifestation rests with the interplay of T and B cells, as well as factors involved in systemic sclerosis.

Education

501 What Are the Pathology Education Requirements for All Clinical ACGME Accredited Programs in an Academic Center?

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Background: The Accreditation Council for Graduate Medical Education (ACGME) recognizes that knowledge of pathology is integral to the practice of medicine and mandates pathology education for many accredited programs. At academic centers, the pathology department commonly provides this required education for clinical trainees. This study determines the institution-wide pathology education GME requirements (exclusive of pathology residency and fellowships) in an academic center.

Design: Key word searches for "pathology," "laboratory," "autopsy," and "morbidity" were performed on all clinical Residency Review Committees (RRC) program requirements documents. A determination was made as to whether a pathology education requirement was identified for each search occurrence. Requirements were categorized.

Results: ACGME lists 102 clinical programs; 74 exist at Duke. Fifty-six (76%) programs had requirements.

Table 1. Pathology education requirements for clinical ACGME accredited programs at a single academic center.

Pathology Education Requirements	Number(%)
Consultation/Support	29(40%)
Teaching/Conferences	37(50%)
Clinico-pathological correlation	4(5%)
Required rotation	1(1%)
Elective rotation	7(9%)
Clinical resources	3(4%)
Gross/Micro examination	16(22%)
"Residents assigned to the department of pathology"	1(1%)
"Shared experiences with pathology residents"	1(1%)
Obtain copies of autopsy reports	11(15%)
Other	15(20%)

Teaching/conferences (didactic, morbidity/mortality, tumor board, grand rounds, and multidisciplinary) were the most common requirement. Sixteen programs must perform gross/microscopic examination; ophthalmology requires 36 hours. Medical genetics trainees have a required pathology rotation. Elective rotations should be available for neuromuscular disease, child neurology, general surgery, neurological surgery, otolaryngology, and radiation oncology. Others included: collaborations with pathologists (11;15%), "access to microbiology laboratory" (1; 1%), and structured experiences in blood banking and tissue banking" (1;1%).

Conclusions: Pathology departments at academic centers are faced with significant institution-wide pathology education requirements for clinical ACGME programs. Studies are ongoing to determine the hours required to fulfill these mandates (estimated at Duke as 4,500+ hours) and to develop innovative educational tools to satisfy the requirements and make efficient use of pathology teaching faculty.

502 Partners in Pathology: A Model for Department-Wide Educational Experience

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Background: Disease variety in large, tertiary care hospitals is often dictated by the demographics in which these hospitals operate. In order to broaden the overall educational experience in these settings, drawing upon clinicians in other settings must be pursued. Partners in Health is a comprehensive, community-based healthcare organization with clinics in seven countries. Although the disease burden in these settings is primarily infectious, the variety amongst benign and malignant neoplasms is diverse. The collaborative effort between our Department and Partners in Health serves an important role in alleviating health disparities for underserved patients and provides an overlooked opportunity to diversify and broaden educational experiences.

Design: To establish collaboration with clinicians working on-site, to demonstrate the ease of providing pathology services in resource poor settings, and to evaluate the exposure of our staff to different pathology.

Results: Sixty-four consecutive cases from Haiti, Rwanda, and Lesotho from patients with a median age of 27 (range 1-81) were examined. Diagnoses included twenty-five malignancies, eight infectious or inflammatory cases, and other rare entities. The malignancies included two uncommon cases of Kaposi sarcoma with lymphangiomatoid features, a case of malignant teratoid medulloepithelioma, and a case of extranodal sinusoidal histiocytosis. In addition, multiple EBV-associated neoplasms were demonstrated. Among the infectious cases were two cases of tuberculosis, a case of chromoblastomycosis, a case of actinomycosis, and several cases with necrotizing granulomas where no organisms were identified. These cases were frequently presented at various department-wide conferences.

Conclusions: The diversity in non-infectious and infectious diseases represented an invaluable educational experience for our Department. In this cohort, many of the malignancies have been rarely reported in literature. These unusual cases served to educate both our trainees and our senior staff. The collaboration between an academic pathology department and clinicians operating in these resource poor settings was simple and brought with it a host of benefits to both parties.

503 High-Tech or High-Time to Re-Construct? What Type of First Impression Is Your Program Website Giving Prospective Applicants?

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Background: Previous studies have demonstrated that pathology residency applicants obtain much of their information, and thus the first impression of a program, from institutional websites. This study examined pathology program website accessibility and usefulness, in particular, features that may be alluring to prospective applicants.

Design: Current programs and web addresses were obtained from the Fellowship and Residency Electronic Database (FREIDA) list of graduate medical education programs under the pathology specialty section. The hyperlink from the database was used to access individual program websites. An average of fifteen minutes was spent per webpage, assessing fifteen individual content features, ranging from salary and curriculum, to interactive blogs and streaming video.

Results: 86.0% (129/150) of the programs had functioning web pages accessible via database hyperlink and 98.4% (127/129) had a detailed program description. The most common features were an outline of educational curriculum 89.9% (116/129), faculty biographies 68.2% (88/129), listing of current residents 64.3% (83/129), Electronic Residency Application Site (ERAS) link 63.5% (82/129), description of research opportunities 62.7% (81/129), and admissions requirements 62.7% (81/129). The least commonly seen features included resident evaluations 12.4% (16/129) and date/time stamps. 40.3% (52/129). 33.3% (43/129) included high-tech or interactive features such as blogs and streaming video.

Conclusions: Technology and the internet are prevalent in medical and resident education. Pathology programs need to demonstrate an interest and commitment to technology through high (100%) internet accessibility, as well as informative and stimulating site content designed to heighten applicant interest.

504 Digital Games Designed for Pathology Education

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Background: Computer-savvy 'net generation' students think and learn in different ways. Incorporation of such technologies within the confines of their education will enhance their educational experience. Digital gaming is an effective, enticing and encouraging way of learning. As pathology teaching hours compete for continued curricular representation, specially designed digital games for pathology education offers an alternative resource to complement the traditional teacher-led activities for the extended learning environment.

Design: Principles underlying game design construction included components of strategic thinking, interpretative analysis, and problem solving thereby contextually bridging the gap between the theory and application of pathology in a fun, entertaining game-like atmosphere. Use centered games were constructed with high time-on-task activities with motivation/goal orientation through rewards, clues, hints and partial solutions to keep progressing and self directing their own learning.

Results: Three educational games were designed with varying levels of complexity for the 'new' incoming first year medical, dental and physical therapy students and second year medical students. Game#1 – WORD SCRAMBLE AIM: To familiarize, improve vocabulary and gain expertise in the 'new' language of pathology. Game#2 – THE PATH IS RIGHT AIM: To provide a selected review sampling of midterm exam test materials in a fun game like atmosphere Game #3 – PATH TO SUCCESS Aim: To provide a comprehensive review sampling of final exam test materials in a fun game like atmosphere. 10 modified questions from this pool were evaluated in the written examination. Feedback was obtained via a semi-structured survey questionnaire. The notion of an electronic game format as an exam review was well received by students

who found this a useful and effective review tool. They appreciated the variety of game presentations and enjoyed reviewing the material at their convenience individually or in groups. The examination performance outcome of the game content related questions were superior in comparison to the responses in the remainder of the examination.

Conclusions: Such specially constructed educational digital game activities fosters an improved and facilitated, non threatening learning environment with increased student engagement. There is increased accessibility to the information and ease of distribution with accessibility for self directed learning to occur anywhere as learning is often an 'unplanned' experience.

505 Interactive Spaced Education Versus Web-Based Modules for Teaching Histopathology Diagnostic Skills to Urology Residents: A Randomized Controlled Trial

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Background: Although genitourinary (GU) histopathology is core content on the urology board exams, urology residents receive little training in it. Interactive spaced education (ISE) is a novel email-based educational tool based on 'spacing effect', the psychological finding that educational encounters which are spaced and repeated over time result in more efficient learning and improved retention. We performed a randomized trial to compare the efficacy and acceptability of ISE to bolus web-based teaching (WBT) modules as methods to teach GU pathology to US urology residents. **Design:** ISE was delivered using emails that contained pathology images and multiple-choice questions. Upon submitting an answer to a question, residents were presented the correct answer and explanations for all answers. Forty validated questions with images were constructed on testis, prostate, kidney and bladder pathology (10/organ). All US urology residents were eligible to participate. Enrollees were randomized to 1 of 2 cohorts. Cohort 1 residents received 3-cycle ISE on prostate-testis (weeks 1-16) and 3 WBT modules on bladder-kidney (weeks 14-16). Cohort 2 residents received 3-cycle ISE on bladder-kidney (weeks 1-16) and 3 WBT modules on prostate-testis (weeks 14-16). The ISE intervention was delivered via daily emails each with 1 question. The ISE material was distributed in 3 cycles or repetitions to take advantage of the spacing effect. Each cycle was 4-weeks long and consisted of 20 questions with unique images. The WBT used the identical content and delivery system, with the questions aggregated into 3 20-question modules. To assess long-term learning, residents were randomized to complete a final 4-week cycle of 40 questions with unique images covering all 4 organs at 1 of 4 time periods during weeks 18-45.

Results: Seven-hundred twenty-four urology residents enrolled. ISE and WBT were completed by 77% and 66% of residents, respectively. ISE generated a median 15% improvement in long-term knowledge (IQR 5to10; Cohen effect size 1.06), while WBT generated a 5% improvement (IQR -5to15; effect size 0.24; $p < 0.001$). Of 596 survey respondents, 77% preferred ISE over WBT, and 99% requested to participate in future ISE programs.

Conclusions: ISE is an effective and well-accepted method to teach GU pathology to urology residents. ISE can be utilized to teach pathology diagnostic skills to pathology residents and attendings across the US.

506 Improving Resident Education through Large Volume Surgical Pathology Case Rapid Review

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Background: Pathology residency training programs lack standardization in the methods by which residents are trained to examine and interpret slides. Historically, residents in our program preview a limited number of slides and are mainly observers in case sign-out. We implemented a process whereby senior level residents performed rapid review of large numbers of surgical pathology cases and evaluated the effect on learning and diagnostic accuracy.

Design: A senior level resident rapidly previewed all surgical pathology cases (i.e., < 2 minutes per biopsy and < 15 minutes per large case) and made an interpretation prior to passing the case to the pathologist-junior resident team. We correlated the pathologist-senior resident diagnosis and classified discrepancies as major and minor, based on a subjective assessment of the effect on patient care. We compared performance at the beginning and end of a 3 month rotation and determined anatomical site diagnostic weaknesses. We evaluated how pathologist variation in the use of standardized diagnostic categories (e.g., cervical intraepithelial lesion system) affects resident performance.

Results: In a 3 month rotation, a senior resident previewed, on average, 1950 cases with 2630 case parts. The proportion of resident correct diagnoses and major and minor discrepancies at the beginning and the end of the rotation was 66.5%, 14.3%, and 19.2% and 87.4%, 5.9%, and 6.7%, respectively ($P < 0.05$). Greatest levels of improvement were observed in gastrointestinal pathology (decrease of total case error proportion from 36.3% to 11.6%) and prostate pathology (decrease of total case error proportion from 25.0% to 4.7%). Senior residents had considerably higher levels of discrepancy with some pathologists than others even taking into account the overall improvement over time (e.g., variation from 11% to 17% major discrepancy). The root cause of this variation arose from the lack of pathologist standardization in diagnostic areas with the senior resident adopting criteria from some faculty and exposing differences in criteria use among faculty.

Conclusions: We conclude that large volume surgical pathology rapid review rotations markedly improve the quality of senior level resident diagnostic accuracy and identify specific organ system diagnostic weaknesses. Our study also illustrates that the lack of pathologist diagnostic standardization undermines residency education and exposes unaddressed errors in pathology services.

507 Gender Differences in Preferences for Glass Slides vs. Virtual Slides for Histopathology Testing

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Background: Virtual slide telepathology has many applications including its uses for pathology diagnostic services and for medical education. The University of Arizona College of Medicine switched from using glass slides to virtual slides for teaching pathology during the 2006-2007 academic year. Students had equal exposure to glass slides and virtual slides that year. Virtual slides were scanned with a DMetrix DX-40 ultrarapid virtual slide scanner.

Design: At the end of the academic year, second year medical students were assessed three ways: a timed 150 question written exam; a 60 question projected practical exam; and a 15 minute oral exam. For oral exams, students' met one-on-one with a pathology faculty member. They were given the choice of being examined on glass slides or on virtual slides, at the beginning of the session. The choice was not announced before hand.

Results: 111 second year medical students (57 females and 54 males) took the oral exam. In total, 43 students selected glass slides and 68 chose virtual slides (scanned from the same set of glass slide unknowns). Given the choice of being tested on virtual slides or glass slides, 52.6% of the female students selected virtual slides, whereas 70.4% of the male students selected virtual slides. The difference was statistically significant ($X^2 = 6.69$, $p < 0.01$). Interestingly, oral exam scores were not significantly different for the two modalities. Furthermore, there was no significant difference in the choice of test modality for the students who received pass grades vs. those who received honors grades ($X^2 = 1.252$, $p > 0.05$).

Conclusions: The majority of both female and male medical students preferred to be examined on pathology virtual slides, when given the option. However, the preferences of male students were heavily weighted toward virtual slides whereas female student choices were nearly equally divided between virtual slides and glass slides. These preferences might be reflected in the rate of acceptance of virtual slides by pathologists of different genders.

508 Histopathology Glossary: Evaluating Accessibility to Definitions of Descriptive Histopathology Terms in Textbooks and Internet References Commonly Used by Pathology Residents

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Background: Descriptive histopathology terms are commonly encountered by pathology residents at departmental and national conferences, in the literature, and while signing out cases with attending pathologists. The meanings of these terms are often not self-evident. For proper understanding, a beginning pathology resident must then seek out the definitions in available reference textbooks or internet search engines/websites. Often a considerable amount of time is spent searching through multiple books and/or websites to find a certain term, if it can be found at all. In this study, we evaluated the accessibility to find the definition of many commonly used descriptive histopathology terms in current pathology textbooks and internet sources to better determine if the educational needs of trainees are being met.

Design: 14 pathology references in total were evaluated including 11 commonly used pathology textbooks, and 3 well known internet search engines/websites. 100 descriptive histopathology terms were searched for in the index and then cross-referenced to determine if the respective definition was present in the text. For internet sites, terms were entered into the search line and the definitions were obtained from following links to the first 20 hits. Percentages of the overall availability of terms present were calculated, as well as the percentage for each single reference.

Results: Of 100 pre-determined histopathology term definitions, only 30.8% were available over the 14 references used in this study. When using strictly textbooks, 29.3% of the definitions were found. With internet searches, definitions were available 36.3% of the time. One internet search engine provided the highest overall availability with 67%. The highest accessibility percentage amongst a single textbook was 61%.

Conclusions: Our results indicate that residents must spend a significant amount of time searching the literature to access definitions of commonly used descriptive histopathology terms. No single source holds a large majority of terms on our list. Although these terms are mentioned frequently in the literature and in practice, the true meaning may not be clear to the inexperienced trainee after consulting multiple sources. We believe there is an educational need for sources to more completely define the meaning of these commonly used descriptive histopathology terms, many of which serve as a "word picture" to characterized histologic findings.

509 Resident Quality Improvement Education as a Means To Transform Program and Service Quality

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Background: Quality improvement through teamwork and rapid innovation is increasingly being used in healthcare to redesign existing systems to improve quality, efficiency, and cost effectiveness. In 2008, we initiated a quality improvement track in our residency program as a means to educate residents about quality assurance and implementation and to improve the quality of the diagnostic services in our department. We measured the effectiveness of their projects.

Design: Our educational residency track involves initial immersion through didactic material followed by the selection of a project led by a senior level resident. Initial education consists of a 4-hour Lean course attended by all residents followed by a 4-day Lean course for select residents. In both courses, residents learn the fundamentals of Lean methods and implementation. A senior resident then leads a resident team in root cause analysis, practice redesign, implementation, and analysis.

Results: In 2008, the resident team undertook the problems of less than optimal surgical pathology turn-around time (TAT) and resident dissatisfaction with surgical pathology teaching. Root cause analysis showed a lack of standardization in service schedules and practices in all units (e.g., gross room, histology, transcription). The residents redesigned the service in a step-wise fashion by altering service schedules and duties of faculty, support staff, technicians, and trainees. All changes occurred through group-oriented sessions attended by all personnel. TAT was tracked prior to and during the implementation process. The team first created a service in which more pathologists signed out cases followed by a service in which fewer pathologists signed out cases. Prior to implementation, the percentage of cases signed out in 2 and 3 days was 61% and 77%, respectively. In the initial implementation, when more pathologists were added to the service, the percentage of cases signed out in 2 and 3 days was 80% and 90%, respectively. In the second phase, with a reduction in the number of pathologists, the percentage of cases signed out in 2 and 3 days remained unchanged. All residents showed high levels of satisfaction with the redesigned services.

Conclusions: Our residency quality improvement educational program was effective in improving quality and in leading residents in transformational change processes. As senior residents are involved in almost all front-line activities and are familiar with the system, their education and process involvement is critical in improving healthcare quality.

510 Diagnostic and Educational Support to Pathologists in the Developing World through Telepathology

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Background: The practice of pathology in the developing world presents challenges in terms of limited resources, shortage of trained personnel and lack of continuing education programs. Telepathology holds great promise as a way to offer diagnostic support, 2nd opinions and ongoing training. We report our experience with a recently established static telepathology program between 2 hospitals in the United States and Tanzania, a country of 38 million people served by only 15 pathologists without access to immunohistochemistry (IHC) or molecular diagnostic testing.

Design: An Olympus BH-2 6-headed microscope and SPOT Insight digital camera were donated to an 80-bed Tanzanian multispecialty hospital with an average specimen volume of 800 cytology & surgical pathology cases/year. 2 local pathologists were given on-site training on image acquisition. Images were uploaded to the iPath open source telepathology server. A US pathologist reviewed images in consultation with subspecialist colleagues, in order to provide both diagnostic and educational support to submitting pathologists as the program's main objective.

Results: During the initial 5 months of the program, 15 cases were submitted for 2nd opinion consultation in subspecialty areas of cytology (4), GI (4), breast (2), head & neck (2), hematology (2) and soft tissue (1). Static images enabled a complete or partial diagnosis in 12 cases (80%). 4 entirely diagnostic cases included a soft palatal pleomorphic adenoma, a breast giant hamartoma, a rectal tonsil and a rectal cavernous hemangioma. 4 partially diagnostic cases were favored to be malignant and 4 were favored to be benign/reactive. Among the 8 partially diagnostic and 3 non-diagnostic cases, factors precluding a definitive diagnosis included absence of confirmatory IHC/flow cytometry (1 soft tissue, 2 hematology cases), air-drying (4 cytology cases), tangential tissue sectioning (1 head & neck case) and non-technical issues (3 cases). Responses posted to completely and partially diagnostic cases included a diagnosis, discussion of the differential diagnosis and additional information (e.g. gross findings, diagnostic pitfalls and/or publications about the entity).

Conclusions: Telepathology is well-suited to provide both diagnostic support and continuing education to pathologists in the developing world. Factors precluding a definitive diagnosis are mainly technical and can be overcome by additional training or building local capacity for basic ancillary testing.

Endocrine

511 Geographical Mapping of a Multifocal Thyroid Tumour Using Genetic Alteration Analysis & miRNA Profiling

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Background: Papillary thyroid carcinoma (PTC) frequently presents as multiple tumour foci within a single thyroid gland or pluriform, with synchronous tumours comprising different histological variants, raising questions regarding its clonality. Among genetic aberrations described in PTC, BRAF V600E mutation and ret/PTC activation occur most commonly. Several studies have investigated the genetic alteration status of multifocal thyroid tumours, with discordant results. To expand on the question of clonality the objective of this study was to examine disparate geographical and morphological areas from a single PTC for the presence of ret/PTC or BRAF mutations and correlate it with miRNA expression profiles.

Design: A multicentric PTC containing classic PTC, insular and anaplastic foci, & tumour cells adjacent to vascular invasion and lymphocytic infiltrate was examined for the presence of ret/PTC & BRAF mutations. Geographical data was correlated with expression profiles of 330 miRNAs. Hierarchical clustering analysis of the profiles, miRNA gene target prediction, & immunohistochemistry were also performed.

Results: Each morphological area proved negative for ret/PTC 1 rearrangement. Two distinct foci with classic morphology harboured the BRAF mutation. All other regions, including the insular and anaplastic were negative for the mutation. MiRNA profiles were found to distinguish tumours containing the BRAF mutation from the other tumour

types, & differentiate between insular and anaplastic tumours. Profiles also included miRNAs previously discovered in this cancer, and miRNAs linked to various processes involved in tumour growth and proliferation.

Conclusions: The initial genetic alteration analysis indicated that pluriform PTC did not necessarily evolve from classic PTC progenitor foci. MiRNA profile analysis, however provided an interesting interpretation to the answer of the clonality question. The hierarchical clustering analysis indicated that the multiple foci may not have arisen due to the clonal metastasis of tumour cells or of independent mutational events, but perhaps both phenomena can occur simultaneously within the one tumour to enhance cancer progression. Putative gene targets were also obtained for the differentially regulated miRNAs raising the question as to the utility of these RNAs as either biomarkers or biological mediators.

512 Pancreatic Specific Transcription Factors and CK19 in Pancreatic Endocrine Tumors

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Background: The role of transcription factors (TFs) *Isl-1, Pax6, Nkx2.2, Nkx6.1, MafB and Pdx-1* in pancreas development was recently described. They regulate commitment to individual cell lineages and maintain terminally differentiated phenotype. Their disruption results in impaired development of pancreatic endocrine structures. Few data are available about their expression in pancreatic endocrine tumors (PETs). PETs are classified in WHO categories as tumors with benign behaviour (WDET-B), uncertain behavior (WDET-U), well differentiated carcinomas (WDEC) and poorly differentiated carcinomas (PDEC). No absolute histopathological criteria are available to predict clinical course. Therefore, identification of immunohistochemical markers that could predict the biological behavior would be extremely helpful in surgical management and adjuvant therapy of PET. The expression of the intermediate filament cytokeratin 19 (CK19) has been recently proposed as predictor of survival in PETs.

Design: To evaluate in a large series of PETs the expression of TFs (*Isl-1, Pax6, Nkx2.2, Nkx6.1, MafB, Pdx-1*) and CK19 and analyze their correlation with WHO categories. TFs expression was immunohistochemically evaluated in a series of 131 PET (48 WDET-B, 32 WDET-U, 41 WDEC and 10 PDEC); TF score was defined as follows: Low (LS), 0-3 TFs immunopositive per case; High (HS), 4-6 TFs immunopositive per case. The same series was evaluated for CK19 expression and its correlation with WHO categories and TFs expression investigated.

Results: HS for TFs (4 or more) was observed in 90% of WDET-B, in 69% of WDET-U, and in 49% of WDEC; all PDEC showed LS ($p < 0.05$). *Nkx6.1* was the most frequently TF associated with WDET-B (79%) in contrast to WDET-U with 50% of positive cases. Low reactivity was observed for *Nkx6.1* in WDEC (20%) and PDEC (10%) ($p < 0.05$). CK19 expression was higher in WDEC (83%) than in WDET-B and WDET-U tumors (56%) ($p < 0.05$).

Conclusions: Endocrine specific TFs are coexpressed in most WDET-B and WDET-U; on the contrary, a progressive loss of expression is detected in WDEC and PDEC: the TFs biological role in pancreatic endocrine specification is maintained in PET and run in parallel with WHO PET categories. Among these TFs, *Nkx6.1* is the most sensitive marker of tumor differentiation, with very limited reactivity in WDEC and PDEC. These changes in TFs expression in PET are inversely correlated with CK 19 expression. The combined analysis of TFs and CK19 could offer a better prognostic index in the clinico-pathologic evaluation of PET.

513 Gene Expression Profiles in Archival Thyroid Carcinoma Using Pre-amplification RT-PCR, Immunohistochemistry and MicroRNA Expression Analysis

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Background: Thyroid cancer is the most frequently occurring endocrine malignancy, with world wide thyroid cancer incidence rates increasing year after year. Furthermore thyroid nodules are a common occurrence and distinguishing benign specimens from malignant can be problematic. The development of a robust molecular expression signature encompassing transcriptomic, protein and microRNA expression analysis would greatly aid in the diagnosis of thyroid malignancy.

Design: Two hundred and five formalin fixed paraffin embedded (FFPE) thyroid samples were laser capture microdissected and a novel pre-amplification technique was used to facilitate gene expression analysis using TaqMan® Q-RT-PCR of a panel of 5 targets including MMP11, BAX, APOE, TOP2A and LYN plus endogenous control. A tissue microarray (TMA) was constructed using the same two hundred and five samples and immunohistochemical analysis was carried out for MMP11, BAX, APOE, TOP2A and LYN. Expression of microRNA let-7a was analysed in one hundred and four FFPE thyroid tissue samples using a novel *in situ* (ISH) hybridisation technique for miRNA expression in archival samples.

Results: All five targets were found to significantly ($p < 0.05$) discriminate a range of benign from malignant thyroid disease states at mRNA and protein expression levels. Four out of the five targets were also shown to significantly differentiate, at the gene and protein level, corresponding groups of matched samples. Of these four targets, MMP11 and APOE, which were over-expressed in malignant tissues, were found to be targets of let-7a using miRNA target prediction databases. Expression of let-7a was significantly ($p < 0.05$) down-regulated in all malignant and neoplastic groups, and subsequently was also found capable of discriminating benign from malignant thyroid disease.

Conclusions: This robust panel of molecular markers could prove extremely useful in assisting the classification and diagnosis of thyroid malignancy.