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[1905] A Comparison of Targeted Next Generation Sequencing from Paired Formalin-Fixed and Fresh Frozen Specimens

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- **Background:** Next-generation sequencing (NGS) has the potential to revolutionize molecular testing of cancer through comprehensive, unbiased, and inexpensive mutation detection. However, for these methods to be practical they must be robust to a diversity of specimen types, including formalin-fixed diagnostic tissue. We evaluated the performance and quality of targeted NGS in a series of paired formalin-fixed and fresh-frozen tissue to determine if formalin-fixed tissue is an adequate substrate for these platforms.
- **Design:** Sequence data were generated for 15 pairs (30 specimens total) of case-matched fresh-frozen and formalin-fixed lung adenocarcinoma specimens. Indexed sequencing libraries were prepared with 1ug of DNA that passed ladder amplification and spectrophotometry quality-control assessment. Enrichment for 28 target genes was performed with custom capture reagents and enriched libraries were sequenced by multiplex sequencing. Variants were identified from mapped reads using a custom pipeline based on the Genome Analysis Toolkit. Read quality metrics and single-nucleotide variant calls were compared between sample types. For a subset of cases, calls from sample pairs were compared to previously-generated array-based genotype data.
- **Results:** Multiplex sequencing produced 2,000-10,000-fold coverage of the target region across all samples. There were small, but statistically-significant differences in insert size (179 bp (fixed) vs 225 bp (frozen); $P<10^{-7}$), aligned bases per read (96 bp (fixed) vs. 98 bp (frozen); $P<10^{-5}$), and the fraction of unique reads (22% (fixed) vs 31% (frozen); $P<10^{-3}$). However, the mean number of high-quality bases per read, mean error rate, and proportion of positions with high-quality basecalls were not significantly different. Basecall concordance between paired samples was >99.99%, with an average of only 5 discordant positions over the ~300 kilobase-pair target interval per case. The concordance between basecalls and array-based genotypes was >98% for each specimen type.
- **Conclusions**: We have shown that next-generation sequencing using DNA from formalin-fixed specimens yields comprehensive and accurate sequence information that is comparable to that from fresh-frozen tissue. These results indicate that NGS technology can be readily integrated into the standard surgical pathology workflow and demonstrate the feasibility of large-scale sequencing of formalin-fixed surgical pathology specimens for basic science, translational research, and clinical trials.

  Category: Special Category - Pan-genomic/Pan-proteomic approaches to Cancer

[1912] Alignment in a SNAP: Cancer Diagnosis in the Genomic Age

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- **Background:** As the cost of DNA sequencing continues to drop at a pace exceeding that of Moore's Law, there is growing need for tools that can efficiently analyze ever larger bodies of sequence data. By mid-2013, it is estimated that we will reach the $1000 genome. The cost of sequencing a person's genome will then enter the realm of routine clinical practice and it is expected that each cancer patient will have their genome and their cancer's genome sequenced. In order to assemble and interpret this information from the massive numbers of short reads generated by current sequencing machines, significant technological advancement is necessary. Here, we address the first step in the interpretation of a cancer genome from raw sequence information: sequence alignment.
- **Design:** We tested SNAP (Scalable Nucleotide Alignment Package) against the most popular short read aligners, including BWA, Bowtie, and SOAP. Trials included generation of reads from the hg19 build of the human genome with simulated mutations, insertions, and deletions. Additional trials demonstrating superior performance against longer reads and actual whole genome sequencing data sets will be presented at the conference.

- **Results:** SNAP significantly outperforms existing aligners in terms of speed while achieving higher accuracy.

Comparison of Aligners using 125bp Simulated Single End Reads			
Aligner	Seconds per Million Reads	Accuracy (%)	False Positive (%)
bowtie*	1966	88	0.07
BWA*	3021	93	0.05
MAQ*	17506	92.7	0.08
SOAP2*	555	91.5	0.17
SNAP	10	94	0.05

* These numbers were previously published in [Li et al. Bioinformatics Vol. 25 no. 14 2009, pages 1754–1760]

- **Conclusions:** Currently, aligning a single genome takes roughly 1000 processor hours. We demonstrate a new algorithm and software package called SNAP, which is capable of aligning a genomic dataset consisting of up to 3 billion 100bp reads in 1 hour on a machine rented from Amazon for $2. This is a 100X improvement over current technologies with greater accuracy, higher error tolerance and better performance on longer read lengths, making the package compatible with upcoming developments in sequencing technology. Additionally, SNAP can align against a consensus of genomes rather than a single sequence, allowing it to more effectively discriminate between hereditary sequence variation and somatic mutations. Using SNAP, we can begin to realize the benefits of large sequencing projects such as the TCGA, and to translate their results into personalized therapeutic recommendations for each patient.

  Category: Special Category - Pan-genomic/Pan-proteomic approaches to Cancer

[2152] Next-Generation Pathology: Deep DNA Sequencing and Targeted Therapy for Cancer

Christine Sheehan, Alex Parker, Mirna Jarosz, Sean Downing, Roman Yelensky, Doron Lipson, Gary Palmer, Maureen Cronin, Jeffrey Ross. Albany Medical College, Albany, NY; Foundation Medicine Inc., Cambridge, MA

- **Background:** Gene sequencing currently used to select therapy in non-small cell lung cancer (NSCLC), colorectal cancer (CRC) and melanoma (MM) employs traditional standard-of-care (SOC) “hotspot” single gene mutation analysis. Massively parallel (next-generation sequencing (NGS)) has now been adapted to formalin-fixed (FFPE) specimens to provide high sensitivity detection for point mutations, insertion/deletions, translocations and copy number alterations (CNA).
- **Design:** FFPE specimens from 83 solid tumors (50 CRC, 29 NSCLC, 4 MM) with results available from SOC genotyping by allele-specific PCR (KRAS codons 12/13, EGFR exons 17-20, or BRAF V600E) were fully sequenced for 145 genes by NGS. Hybridization-capture of 2574 exons across 145 oncogenes, tumor suppressor genes and ADME-related genes was performed to produce libraries appropriate for paired-end sequence analysis on the Illumina HiSeq2000 platform (Illumina, Inc., San Diego, CA).
- **Results:** NGS recapitulated the SOC test results in all cases. In-depth sequence analysis with median coverage averaging 213-fold (range 8 to 461) detected a per-sample average of 2 previously-described mutations, 7 novel mutations and 2 CNAs in the CRC, including frequent alterations in *TP53* (33), *APC* (27), *KRAS* (12) and *BRAF* (6). The NSCLC averaged 1 previously described mutation, 8 novel mutations and 1 CNA per sample, most frequently *KRAS* (10), *TP53* (7), *JAK2* (3), *EGFR* (2) and *BRAF* (2). The MM exhibited on average 1 previously described mutation, 7 novel mutations and 3 CNAs including *TP53* (4) and *BRAF* (2). In addition to validated clinically actionable mutations in EGFR, KRAS, and BRAF, and multiple alterations in well-known cancer genes (*TP53, STK11, APC, MLH1, BRCA2, SMAD4*), a significant number of additional genomic alterations that have potential therapeutic implications were also detected including activating mutations in the PI3 kinase subunit gene *PIK3CA*; mutations in *MET, KIT, ERBB2* and *CDKN2A*; driver mutations not usually associated with solid tumors, such as the lymphoma-associated *JAK2* V617F mutation in two NSCLCs; and in 1 CRC, a novel chromosome 2 rearrangement adjacent to the *ALK* kinase domain confirmed by analyzing a cDNA library constructed from extracted tumor RNA.
- **Conclusions:** NGS of hundreds of cancer-related genes can be reliably performed at a high level of sensitivity and specificity in clinical FFPE samples of solid tumors, can reproduce SOC single gene traditional sequencing results and shows great potential to inform on therapeutic decisions for patients with CRC, NSCLC and MM.

  Category: Techniques

[1947] Identification of Pathogens in Archival Tissues Using a High-Throughput Sequencing Approach, 3SEQ

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- **Background:** The extent to which viruses and bacteria are related to chronic disease and neoplasia remains questionable. Next generation sequencing (NGS) offers a promising tool for identifying RNA and DNA from viruses and bacteria in human tissue. 3SEQ, a type of RNA-seq requiring only 3' ends, was recently described as an NGS method for gene expression profiling of archival pathology tissues (Beck AH et al, 2010).
- **Design:** We performed 3SEQ and evaluated for candidate non-human genetic sequences in 193 formalin-fixed paraffin embedded (FFPE) samples from the pathology archives. The tissue samples included a wide range of neoplastic, non-neoplastic disease, and normal specimens. Sequences that did not map to the human genome, and passed a filtering step to remove low-complexity reads, were compared with 3752 viral genomes (virome) and 1016 bacterial genomes (bacteriome). Following alignment of the candidate non-human reads to the virome and bacteriome, peak calling was performed to identify regions enriched for sequence reads that likely represent microbial transcripts within the human tissue specimen.
- **Results:** From the 193 FFPE samples, 2.9 billion 36-bp sequence reads were obtained using 3SEQ. Of these, 222 million candidate non-human reads were identified and compared to the virome and bacteriome. This analysis not only allowed us to identify viral and bacterial sequences in FFPE tissue samples, but also to characterize expressed transcripts from those genomes. For example, we observed the expression of three Epstein-Barr viral genes in 8 of the 9 nasopharyngeal carcinoma (NPC) samples and were able to quantify their expression across samples. PCR validation and Sanger sequencing was used to confirm the presence of the transcript with the most robust 3SEQ peak. Additional candidate viral and bacterial peaks from various diagnoses are now under investigation.
- **Conclusions:** 3SEQ is a useful tool for exploring pathogen gene expression in a wide variety of human disease. In archival human pathology tissue, the 3SEQ method combined with the peak-calling algorithm increases sensitivity and scope for identifying transcript termini of pathogens within a landscape of incompletely annotated viral and bacterial genomes.

Category: Pathobiology

[1901] A Novel microRNA-Based Test Demonstrate above 90% Accuracy in Classification of Metastatic Tumors from Patients Diagnosed with Carcinoma of Unknown Primary

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- **Background:** Identification of the tissue of origin of metastatic tumor is vital to its management. Carcinoma of unknown primary (CUP) is common in oncology, representing 3-5% of all invasive malignancies. A microarray-based test that measures the expression of 64 microRNAs was employed to identify the tissue of origin of metastatic tumors of CUP cases.
- **Design:** A cohort of resected metastatic lesions from patients diagnosed with CUP was studied. The cohort included 93 samples (from 92 patients) with adequate tissue sample needed for the test. Eight samples failed due to inadequate RNA quality; 85 samples (84 patients) were processed successfully. Test results were compared with clinical presentation including imaging, pathological data (histology and IHC) and therapeutic response.
- **Results:** In this blinded study, the test results were fully concordant with the diagnosis based on all the clinical and pathological information available including follow-up and outcome data in over 90% of the cases. The microRNA test assigned a single putative tissue of origin for 50 samples and two tissues of origin in 34 patients with the first being the more likely diagnosis. When comparing only the first (or single) diagnosis, a concordant level of >83% is achieved. The diagnosis based on the clinical and pathological data that was available at presentation and without additional data gathered throughout patient management had only 70% agreement with the test results. Additional clinical and pathological analysis of the CUP cases is currently ongoing.
- **Conclusions**: In a cohort of metastases from CUP patients, a previously developed test based on the expression profile of 64 microRNAs allowed accurate identification of tissue of origin in the vast majority of the cases. The high accuracy of this test in identifying the tissue of origin of metastasis of unknown primary has been validated by this study and demonstrates its clinical utility. The high concordance of the test results to the final diagnosis of the patient demonstrates the importance of the test to yield additional data valuable for patient's management at an early stage of patient's journey.

Category: Special Category - Pan-genomic/Pan-proteomic approaches to Cancer

[591] EGFR Expression and V600E BRAF Mutations Influence Disease Progression in Thyroid Carcinoma

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- **Background:** The worldwide incidence of thyroid carcinoma is steadily rising with a concomitant increase in disease-associated mortality. Activating mutations in *BRAF* are associated with aggressive disease in papillary thyroid carcinoma (PTC). Epidermal growth factor receptor (EGFR) is an upstream receptor in the BRAF signaling pathway and is overexpressed in anaplastic carcinoma (APC). We investigated how oncogenic mutations in *BRAF* and *EGFR* and EGFR protein expression influenced disease outcomes in the four major thyroid carcinoma subtypes: PTC, follicular (FC), medullary (MC), and APC.
- **Design:** Two pathologists scored 79 cases of thyroid carcinoma from a TMA for EGFR intensity (0–3+) and percent positivity (0–100%). High EGFR expression (EGFR-H) was defined as 3+ staining or 2+ staining of ≥ 50% of cells. DNA was successfully extracted from single paraffin blocks of 59 cases for mutational analysis (MA). Activating *BRAF* V600E (T→A 1799) or *EGFR* exon 21 L858R (T→G 2573) mutations, or *EGFR* exon 19 deletions (del 2235-2249/2236-2250; del E746-A750) were determined using pyrosequencing. The subtypes tested (IHC/MA) included PTC (23/18), FC (35/33), MC (9/2), APC (12/6), and unclassifiable (1/1). Statistical significance was determined using Fisher's exact test.
- **Results:** In PTC, EGFR-H correlated with lymph node (LN) metastases ($p< 0.01$) but not with survival or recurrence. EGFR expression increased the risk of recurrence in Hurthle cell variant of FC ($p= 0.054$). EGFR-H was seen in all APCs (12/12). No EGFR expression was seen in MC (0/9). None (0/59) of the carcinomas demonstrated *EGFR* exon 19 deletions or exon 21 activating mutations. 10% (6/59) of the carcinomas harbored V600E *BRAF* mutations (2 APCs and 4 metastatic PTCs) and all 6 expressed high levels of EGFR by IHC. V600E *BRAF* mutations correlated with LN metastases ($p< 0.01$) and 50% (3/6) of patients with BRAF mutations survived <6 months.
- **Conclusions:** EGFR expression increased the risk of recurrence in Hurthle cell variant of FC, and EGFR-H correlated with LN metastases in PTC. V600E *BRAF* mutations were associated with LN metastases and decreased survival. No E746-A750 deletions or L858R EGFR mutations were identified by pyrosequencing suggesting that alternate mechanisms for EGFR overexpression are involved in thyroid carcinogenesis. The finding that all cases of V600E BRAF mutations coexpressed EGFR-H suggests that in thyroid carcinomas unlike in lung adenocarcinomas, EGFR expression and downstream BRAF activating mutations may not be mutually exclusive.

Category: Endocrine

[1789] Low Rate of *IDH1* R132H Mutation in Adult Non-Supratentorial Low and Intermediate Grade Diffuse Gliomas

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- **Background:** Diffuse gliomas (DG) are most frequent in supratentorial locations; however, they also rarely occur in the brainstem, cerebellum and spinal cord. Minute biopsies from these sites are often challenging to interpret and could benefit from diagnostic ancillary studies. Isocitrate dehydrogenase 1 (*IDH1*) mutation status has been shown to help distinguish adult low grade DG from reactive gliosis or from different CNS tumors with overlapping histologic features; however, published data on *IDH1* mutation status in DG have focused on supratentorial tumors which may not share the same biology as their non-supratentorial counterparts. The cumulative reported rate of *IDH1* R132H mutation in grade II or III DG is up to 75%.
- **Design:** We searched our archives for cases of adult grade II or III DG involving brainstem, cerebellum or spinal cord. Cases with available archived tissue were processed for mutant *IDH1* R132H immunohistochemistry.
- **Results:** Thirty-three cases had tissue available, including 14 from brainstem (6 diffuse astrocytomas (DA) and 8 anaplastic astrocytomas (AA)), 10 from cerebellum (2 DA, 1 low grade glioma and 7 AA) and 9 from spinal cord (4 DA, 3 AA and 2 oligodendrogliomas). The median age at diagnosis was 47 years (range 17-78). Remarkably, only 3 (9%) of 33 tumors were positive for mutant *IDH1* R132H immunohistochemistry, including 2 (20%) of 10 in the cerebellum (2 AA) and 1 (7%) of 14 in the brainstem (1 DA). None of 9 spinal cord tumors were positive for the mutant protein.
- **Conclusions:** In contrast to the high reported rate of *IDH1* R132H mutation in supratentorial grade II or III DG, the rate in non-supratentorial cases appears very low, suggesting location-specific biology in these tumors. Genome-wide studies and mutation profiling are warranted to further explore that possibility and to exclude other *IDH1/2* mutations. The low prevalence of *IDH1* R132H mutation in non-supratentorial DG may limit its use as a diagnostic tool in this setting.

Category: Neuropathology

[1797] Pilocytic Astrocytomas with Infiltrating Patterns of Growth Carry a High Rate of BRAF V600E Mutation

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- **Background:** Pilocytic Astrocytoma (PA), a WHO grade 1 tumor, shows two frequent alterations in BRAF oncogene.The V600E point mutation, which is the most common tumor-associated alteration in the BRAF gene and an alternative BRAF activating mechanism that involves BRAF-KIAA1549 gene fusion with corresponding duplication at chromosome band 7q34. Although the majority of PAs are well circumscribed, a subset shows an invasive pattern akin to biologically different infiltrative gliomas. We investigated whether the presence of a BRAF point mutation and/or duplication in PAs correlate with an infiltrating pattern of growth.
- **Design:** We identified 19 cases of PAs with brain infiltration (5 from NF1 patients) and matched them with 18 localized PAs for a total of 37 cases. Infiltration was assessed by histology and MRI. 26 cases were in adults and 11 in children.

  V600E mutation was identified by an allelic discrimination PCR mutational kit. Corriel Repository HBT-38 cell line was used as a positive control. FISH studies utilized Abbott Molecular kit with probes for both CEP7 (Green) and 7q34 BRAF containing region (Gold). In each case, 50 cells were evaluated. In the cells with two green signals, presence of two gold signals indicated wild type whereas three a duplication. Cases with over 20% of cells with a gain of BRAF were scored positive for duplication. Both tests were done on all cases.

  Statistical analysis was performed using two group Fisher's-exact test of equal proportions. This compared infiltrative vs non-infiltrative tumors with V600E mutation.
- **Results:** BRAF abnormality by either PCR or FISH was seen in 17/37 cases. The V600E mutation and the gene duplication were mutually exclusive except for one case. 6 cases showed a V600E mutation. Strikingly, 5/6 cases with the point mutation showed distinct brain infiltration. 5/14 infiltrating PAs (excluding patients with NF1) had the V600E mutation, whereas only 1/18 non-infiltrating tumors had this pathogenic point mutation. This difference attained a p-value of 0.064 by using Fisher's-exact test of equal proportions. Duplication of the BRAF gene was seen in 12/37 cases. In contrast to V600E mutation, the duplication was non-discriminatory in respect to infiltration of the PAs. Of 5 tumors in NF1 patients, one was positive for duplication and none for point mutation.
- **Conclusions:**

  1. Presence of BRAF V600E mutation appears to correlate with an infiltrating pattern of PAs in non-NF1 patients.
  2. Duplication of the BRAF gene in PA is present in both infiltrative and localized tumors.
  3. BRAF abnormalities are rare in NF1 PAs.

  Category: Neuropathology

[1762] The p53 Negative Regulator *MDM4* Is Amplified and Over-Expressed in Hepatoblastoma

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- **Background:** The p53 tumor suppressor pathway is inactivated in virtually all cancers. In many tumors, amplification or over-expression of *MDM4* and *MDM2* abolish the p53-mediated oncogenic stress response by inactivating the wild-type p53 protein. Restoring p53 function using inhibitors to disrupt the MDM4/MDM2/p53 interactions is a promising new therapeutic strategy.

  Hepatoblastoma (HB) is a highly aggressive neoplasm of childhood. While most HBs are managed with surgery and chemotherapy, no effective treatment exists for refractory and recurrent tumors. Since *TP53* mutations are rare in HB, we posited that a systematic evaluation of *MDM4* and *MDM2* amplification and over-expression in HB may reveal a common mechanism of p53 inactivation and a potential therapeutic target.
- **Design:** Archival specimens (n=26) were obtained with IRB approval. In cases with double minutes (dmins) on cytogenetics, spectral karyotyping (SKY) and array comparative genomic hybridization (aCGH) was used to fine-map the minimum genomic interval. FISH analyses with *MDM4* and a chromosome 1q control probe were performed on 26 FFPE samples. 100 cells were counted and scored as amplification (>5 copies), copy-gain (3-4 copies), chromosome 1 polysomy (>2 copies of both probes), or normal.

  Real-time qPCR for *MDM4*, *MDM2* and p21 was performed on 21 frozen samples. Data in triplicate was normalized to *GAPDH* and plotted as fold-change compared to normal liver. MDM4 expression was evaluated by immunohistochemistry.

- **Results:** In 2 HB cases with dmins, SKY and aCGH analyses mapped to a ~1 Mb region on Chromosome 1q32.1 containing *MDM4*. *MDM4* FISH analysis identified genomic amplification in 2 cases and copy gain in 8 cases (10/26 cases, 38.4%). Five additional cases had 1-2 extra copies of *MDM4* and chromosome 1 polysomy. *MDM4* expression was 2- to 53-fold up-regulated in 8/21 (38%) cases, including two cases without amplification or copy gain. Nuclear MDM4 protein was detected in amplified cases. *MDM2* expression was however increased in only 1 case. Expression of the p53 transcriptional target, p21, was >2-fold down-regulated in 7/21 (33%) cases, suggesting p53 pathway suppression downstream of MDM4 over-expression.
- **Conclusions:** *MDM4* amplification/copy gain and over-expression, as detected by interphase FISH and qPCR, is a common mechanism by which wild-type p53 can get inactivated in HBs. In contrast, *MDM2* over-expression is a rare event in our series. Our current studies are evaluating the efficacy of small molecule inhibitors in restoring p53 function in the HepG2 hepatoblastoma cell line.

Category: Liver

[1282] Human Papillomavirus-Related Carcinomas of the Sinonasal Tract

- **Justin A Bishop, Theresa W Guo, David S Smith, Hao Wang, Sara I Pai, William H Westra. The Johns Hopkins Hospital, Baltimore, MD; Cleveland Clinic Lerner College of Medicine, Cleveland, OH**

- **Background:** High risk human papillomavirus (HPV) is an established cause of head and neck carcinomas arising in the oropharynx. The presence of HPV has also been reported in some carcinomas arising in sinonasal tract, but little is known about the overall incidence of HPV-related carcinomas of the sinonasal tract or the histologic features that characterize these tumors.
- **Design:** We searched the surgical pathology archives of The Johns Hopkins Hospital for all carcinomas arising in the sinonasal tract from 1995 to 2011. We constructed tissue microarrays from 141 of the tissue blocks, and used whole slides for 37 additional cases. In situ hybridization for high-risk types of HPV was performed.
- **Results:** Of 178 sinonasal carcinomas, thirty-five (20%) were positive for high risk HPV. The HPV-related carcinomas occurred in 20 men and 15 women ranging in age from 33 to 87 years (mean 55). HPV-positive carcinomas consisted of 26 squamous cell carcinomas and variants (14 non-keratinizing, 4 papillary, 4 adenosquamous, 3 basaloid, and 1 keratinizing), 1 small cell carcinoma, 1 sinonasal undifferentiated carcinoma, and 7 carcinomas that were difficult to subtype by current classification schemes. These unclassifiable carcinomas were uniformly associated with high grade features (e.g. high mitotic rate, tumor necrosis), a basaloid component, a ductal component reminiscent of salivary gland origin (e.g. tubular and cribriforming structures, stromal matrix deposition), and an immunohistochemical profile demonstrating the biphasic presence of ductal cells (c-kit and cytokeratin positive) and myoepithelial cells (S100, actin, calponin, and p63). Although these tumors were originally diagnosed as salivary gland neoplasms (e.g. solid variant of adenoid cystic carcinoma in 3 cases), the common presence of squamous dysplasia of the lining epithelium suggested surface origin.
- **Conclusions:** The presence of high risk HPV in 20% of sinonasal carcinomas suggests that it may play an important etiologic role for some carcinomas arising in the nasal passages. While non-keratinizing squamous cell carcinoma is the most common histologic type, there is a wide morphologic spectrum of HPV-related disease that includes a previously unrecognized high grade variant that is prone to confusion with salivary gland tumors, particularly the solid variant of adenoid cystic carcinoma.

Category: Head & Neck

[39] Clinicopathological and Prognostic Significance of Akt-mTOR and MAPK Pathways and Antitumor Effect of mTOR Inhibitor in Malignant Peripheral Nerve Sheath Tumor

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- **Background:** Malignant peripheral nerve sheath tumor (MPNST) is a chemotherapy-resistant sarcoma showing a poor prognosis. A novel effective antitumor drug for MPNST is desired, but it has not been found yet. Akt-mTOR and MAPK signaling pathways are known to be activated in various types of cancer, and some kinds of mTOR inhibitors are available in clinical practice. However, the clinicopathological and prognostic significance of activation of Akt-mTOR and MAPK pathways in MPNSTs have yet not been revealed. Additionally, antitumor efficacy of mTOR inhibitor in MPNST has not been investigated well.
- **Design:** We investigated the activation status of Akt-mTOR (Akt, mTOR, p70S6K, S6RP, 4E-BP1, HIF-1α) and MAPK (Erk1/2) pathways in 129 MPNST formalin-fixed paraffin-embedded (FFPE) samples from 99 patients by immunohistochemistry (IHC). Five samples, for which frozen material was available, were also investigated by Western blotting (WB). The antitumor effect of mTOR inhibitor (Everolimus) was examined using 6 MPNST cell lines (FU-SFT8611, FU-SFT9817, HS-sch-2, HS-PSS, YST-1, FMS-1) by CCK-8 cell viability assay, Matrigel invasion assay and wound healing assay.

- **Results:** Immunohistochemically positive expressions of phosphorylated-Akt (p-Akt), p-mTOR, p-p70S6K, p-S6RP, p-4E-BP1, HIF-1α and p-Erk1/2 were observed in 59.7%, 48.8%, 62.8%, 54.3%, 62.8%, 74.4% and 72.9% of MPNST samples, respectively. The expression levels of p-Akt and p-mTOR by WB corresponded closely to the levels observed by IHC. Clinicopathological examination showed that activation of Akt-mTOR and MAPK pathways was frequently observed in the subgroups of deep location, frequent mitoses, high MIB-1 labeling index and high histological grade. Prognostic analysis revealed that activation of Akt-mTOR pathway was significantly associated with poor prognosis, but activation of MAPK pathway did not influence the prognosis. Experiments with MPNST cell lines showed that Everolimus caused concentration-dependent inhibition of MPNST cell proliferation. Everolimus also inhibited cell invasion and cell migration at a lower drug concentration achieved clinically.
- **Conclusions:** Activation of Akt-mTOR pathway is associated with malignant progression and poor prognosis in MPNST. mTOR inhibition by Everolimus shows antitumor effect in MPNST cell lines and can be a candidate therapeutic target in MPNST.

Category: Bone & Soft Tissue

[296] Frequent *PIK3CA* Mutations in Radial Scars

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- **Background:** Radial scars are breast lesions of uncertain pathogenesis that are associated with a two-fold increased risk of breast cancer compared to controls. Activating point mutations in *PIK3CA* are found in 25-30% of invasive breast cancers; however, they have not previously been investigated in most non-carcinomatous lesions. We sought to evaluate radial scars for known activating point mutations commonly seen in invasive breast cancer.
- **Design:** Sixteen surgical cases containing 24 distinct lesions were identified from pathology archives (2002-2010). Radial scars were intimately associated with a spectrum of epithelial morphology; 18 had non-atypical hyperplasia or columnar cell change, five had atypical ductal hyperplasia (ADH) or ductal carcinoma in situ (DCIS), and one had invasive ductal carcinoma (IDC). We also tested metastatic IDC in a lymph node in a patient with an unknown primary who had three discrete radial scars associated with non-atypical epithelium. Lesional tissue was macro-dissected from unstained paraffin sections; genomic DNA was then extracted and screened for a panel of known hotspot mutations using PCR and mass-spectroscopy analysis. The mutation panel covers 643 mutations in 53 genes, including *AKT1/2/3, BRAF, CDK4, CTNNB1, EGFR, ERBB2, FBX4, FBXW7, FGFR1/2/3/4, GNAQ, HRAS, KIT, KRAS, MAP2K1/2/7, MET, NRAS, PDGFRA, PIK3CA, RET, SOS1*, and *TP53*.
- **Results:** Of the 24 lesions, 12 (50%) had *PIK3CA* mutations (11 with exon 20 H1047 mutations and one with an exon 9 E545K mutation). The remaining 12 lesions were wild-type for all of the screened genes. Of the radial scars without epithelial atypia, 9/18 (50%) had *PIK3CA* mutations; furthermore, 3/5 (60%) of radial scars with atypia had mutations detected. The IDC within a radial scar was wild-type. Interestingly, in the patient with three non-atypical radial scars and a positive lymph node, two of the radial scars as well as the metastatic IDC exhibited the *PIK3CA* exon 20 H1047R mutation whereas the third radial scar was wild-type. No other mutations were found with the extensive screening panel.
- **Conclusions:** In this study, 50% of radial scars showed mutations in *PIK3CA*, which is notably higher than the 25-30% mutation frequency of invasive breast cancer. This finding raises interesting questions as to the role of PIK3CA mutations in breast cancer development. Additional larger studies are indicated to confirm and extend these observations in understanding the pathogenesis of radial scars and their relationship to breast cancer.

Category: Breast

[1953] Aurora Kinase Inhibitors as a Novel Targeted Drug Therapy for Bladder Cancer

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- **Background:** Conventional chemotherapy for invasive bladder cancer has limited efficacy and is generally associated with poor patient prognoses. This study aims to evaluate the potential of targeting the aurora kinases, a family of mitotic regulators, with pharmacologic inhibitors as a novel treatment for bladder cancer.
- **Design:** Expression of genes associated with the mitotic spindle checkpoint, including the aurora kinases A and B, in clinical tissue samples of urothelial and squamous cell carcinomas was evaluated by RNA microarray and reverse-transcriptase PCR. Urothelial carcinoma cell lines UM-UC-3 and T24 were treated with the nonspecific aurora kinase inhibitor ZM447439 and the aurora kinase A inhibitor MLN8237, either alone or in combination with gemcitabine or paclitaxel. Effects of drug treatments were evaluated by flow cytometry with propidium iodide staining, immunofluorescence microscopy, MTS proliferation assay, and TUNEL labeling.

- **Results:** RNA microarray analysis comparing human tissue specimens of urothelial (N=8) and squamous cell carcinomas (N=9) of the bladder to normal urothelium (N=10) identified overexpression of 13 gene transcripts related to the mitotic spindle checkpoint, including aurora kinases A and B, in the cancer specimens. Upregulation of these genes was validated by RT-PCR on a separate set of clinical samples (N=4 for each group). Next, we evaluated the impact of aurora kinase inhibitors on the bladder cancer cell lines in vitro. ZM447439 and MLN8237 treatment of UM-UC-3 and T24 cell lines at concentrations of 10 nM to 1 μM induced G2/M cell cycle arrest and aneuploidy (>4N DNA content) in a dose dependent manner. Immunofluorescence microscopy revealed abnormal mitotic figures with multipolar spindle apparatuses in treated cells. Both cell lines also exhibited cell death by positive TUNEL staining 48 hours after treatment with either inhibitor. Simultaneous treatment of T24 cells with MLN8237 and paclitaxel and subsequent MTS proliferation assay revealed an antagonistic interaction between paclitaxel and MLN8237, whereas simultaneous treatment with MLN8237 and gemcitabine resulted in an additive effect.
- **Conclusions:** Several mitotic spindle checkpoint proteins, including the aurora kinases, are overexpressed in bladder cancer. Our data indicate that aurora kinase inhibitors have significant potential as a novel therapy for bladder cancer, as they induce abnormal mitosis, cell cycle arrest, and eventual death of bladder cancer cells in vitro.

Category: Pathobiology

[1958] Characterisation of t(10;17)(q22;p13) in Clear Cell Sarcoma of Kidney

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- **Background:** Clear cell sarcoma of kidney [CCSK], the second commonest pediatric renal cancer is classified as unfavorable histology by the National Wilms Tumor Study group. It is diagnostically challenging, therapy-resistant and has poor outcomes. Nothing is currently known about CCSK biology. Array CGH has shown no consistent genomic aberrations. Three case reports of CCSK with balanced translocation t(10;17)(p13;q22), prompted our investigation into t(10:17), to identify the genes involved and establish translocation incidence.
- **Design:** 51 CCSKs were sourced from Europe and North America, touch imprints for fluorescence in-situ hybridisation [FISH] made and RNA extracted. The breakpoints on chromosomes 10 and 17 were identified in the index case with t(10;17)(q22;p13), using fluorescently labelled BACs. RT-PCR with primers specific for candidate genes within the breakpoint regions was carried out to identify the fusion transcript in the index case and the product sequenced. The 50 CCSKs were screened by FISH and RT-PCR for evidence of the translocation/transcript.
- **Results:** t(10:17)(q22;p13) in CCSK involves YWHAE on chromosome 17 and members of the FAM22 gene family on chromosome 10. Exons 1-5 of YWHAE are fused in-frame to exons 2-7 of FAM22 genes. The YWHAE-FAM22 fusion transcript was identified in 7 of 51 CCSK cases, 12% of the total cases tested. Clinico-morphological correlates of translocation status were investigated and significant vv differences in tumor stage and cellularity noted between transcript-positive and transcript-negative cases.
- **Conclusions:** Identification of the genes involved in CCSK-associated t(10:17) represents the first step in understanding CCSKmolecular genetics. Although the proportion of cases with the translocation is relatively low [12%], it is possible that the involved genes are dysregulated by alternative means in transcript-negative cases. There was a significant difference in stage of transcript-positive versus -negative cases with no transcript-positive case presenting with stage I disease, despite this representing 31% of cases. No significant differences in outcome were detectable between transcript-positive and transcript-negative cases. We are now studying the cell biological effects of expressing this transcript.

Category: Pediatrics

[462] microRNAs as Prognostic Biomarkers in Malignant Melanoma

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- **Background:** MicroRNAs (miRs) are important regulatory molecules. Many recent advances have shown their dysregulation in cancer. Our laboratory has previously reported altered expression of key miRs in melanoma. To develop a further understanding of the clinical importance of such miRs, we have assembled a cohort of primary melanoma patients.
- **Design:** The Agilent miRNA microarray platform was used to generate global miRNA expression profiles for 66 primary melanoma tissue samples. The tumours were from single institution cases, with uniform treatment and follow-up protocols for which clinical data were available. Using supervised analyses, we looked for association of expression level for each miR with pathological (Breslow depth less than or equal to 2mm versus greater than 2mm and low mitotic count versus high mitotic count) and clinical (no metastatic progression versus presence of distant or regional metastasis and alive versus deceased) endpoints.
- **Results:** We identified numerous miRs whose expression level appeared associated with both clinical and pathological endpoints, in most cases showing lowered expression in the more aggressive disease. Of particular interest, we found that expression of miR-150 as well as members of the miR-200 family were significantly associated with the pathological endpoints measured. These miRs were relatively downregulated in thicker melanomas and those with high mitotic rates compared to the thinner or less mitotically active tumours. When clinical endpoints were assessed, lower expression of miR-150 was also strikingly correlated with death and with the presence of metastatic disease.
- **Conclusions:** We conclude that miR expression levels measured in the primary diagnostic lesion can be used to prognosticate clinical outcome in melanoma. The miR-200 family and miR-150 appear to be most promising in this regard. We are currently investigating a role for these miRs and their putative target mRNAs in melanoma progression and outcome, using experimental, statistical and bioinformatics approaches.

Category: Dermatopathology