Scientific Papers

1. Traditional and Electronic Ki-67 Quantitation in Oligodendrogliomas

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The Ki-67 proliferative index has become a useful, objective, immunohistochemical tool that can aid in grading and prognostication for patients with oligodendrogliomas. Previous studies have described the prognostic significance of the Ki-67 index for such patients.

According to the WHO classification of tumors of the central nervous system (2007) "mitotic activity is low in WHO grade II oligodendroglioma, and labeling indices for proliferation markers are accordingly low, usually below 5%". Furthermore, the predictive value of the Ki-67 index appears to be independent of age, tumor site, and histological grade. What is less well described is the relative accuracy of traditional vs. semi-automated methods of enumeration for a test where small differences can influence grading, prognosis and treatment. Tang et al. (2012), studying gastroenteropancreatic neuroendocrine tumours, found high concordance between two semi-automated methods for Ki-67 quantitation whereas "eyeballed estimates" were far less reliable. We will compare the reported proliferative index estimates to those calculated by digital image analysis of 35 recent oligodendrogliomas from the LHSC Pathology archives.

2. SUMO1-CDK6 conjugation drives the cell cycle and retains the self renewal of glioblastoma stem cells

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The current concept in the cell cycle stipulates that constant synthesis without coupled ubiquitin- mediated proteolysis maintains the levels of cyclin-dependent kinase proteins through the cell cycle. CDK proteins are elevated in glioblastoma, which has long been thought due to gene amplifications, although the amplifications occur only in a small set of the tumors according to the genome.

In this study, we show that the G1 phase CDK6 is a substrate of both ubiquitin and small ubiquitin-like modifier-1 (SUMO1), and that CDK6 is sumoylated due to the elevated activity of SUMO1 conjugation in glioblastoma. CDK6 sumoylation at Lys 216 structurally blocks the access of ubiquitination molecules to the Lys 147 ubiquitination site; thus, CDK6 sumoylation stabilize the protein, maintains the kinase activity and drives the cell cycle through G1/S transition. Inhibition of SUMO1 conjugation causes G1 arrest and abolishes the self-renewal and tumorigenic property of glioblastoma initiating or stem cells. In conclusion, SUMO1-CDK6 conjugation constitutes a new mechanism of cell cycle control and selective inhibition of SUMO1 conjugation may provide a novel strategy for the development of the cancer stem cells-targeted treatment.

3. Characteristics of Glioblastoma in Latino Americans

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Latino Americans are a rapidly growing ethnic group in the United States. The characteristics of glioblastoma in this population is poorly studied. We have evaluated the data of 47,540 glioblastoma patients from the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute. This SEER data from 1973-2000 includes up to 13 cancer registries. For 2001 to 2011, the data has improved geographic coverage with 18 registries encompassing 28% of the U.S. population.

Latinos have a lower incidence of GBM than non-Latino Whites. Gender distribution is similar. The total SEER data show that Latinos present slightly younger and have a higher incidence of giant cell glioblastoma and gliosarcoma than non-Latino Whites. Despite higher rates of radiation therapy, the one year survival rate (34.7%) for non-Latino White populations is less than for Latinos (39.0%, $p < 0.001$). Subset analyses (2001-2011) of all the above parameters show similar results except for gliosarcoma incidence. A literature search does not identify MGMT or IDH1 data regarding Latino Americans.

We have assessed 2 prognostic markers in 30 Latino glioblastoma patients. MGMT methylation is present in 24% and IDH1 mutation is found in 12.5%. Our preliminary data suggests that Latinos may have a greater incidence of MGMT unmethylated tumors. Younger age may possibly contribute to improved survival in Latinos but the underlying molecular basis is unresolved.

4. "Biphasic" histology is associated with the non-WNT/SHH molecular subtype of medulloblastoma

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Introduction: In 2007, Ellison et al coined the term "biphasic" medulloblastoma (B-MB) to characterize histology that mimicked the desmoplastic nodular (DN) variant on routine staining, but which lacked internodular reticulin deposition. Via interphase FISH, and utilizing markers for 9q22 and chromosome 17 alterations (ie, -17p and i17q), Ellison et al. suggested that B-MB and DN-MB were genetically different.

Methods: We performed a clinicopathologic review of MBs treated at BCCH from 1986-2011. Using nanoString's n Counter Analysis System (nCAS), each tumor was molecularly subtyped (ie, WNT, SHH, group 3 or group 4). All original glass slides were reviewed to determine WHO histologic subtype [ie, classic, large cell anaplastic (LCA), DN, MB with extensive nodularity (MBEN)]. Tumors were also evaluated for nodularity (scattered vs. frequent) and advanced neuronal differentiation. Reticulin staining was assessed on all cases.

Results: 20 B-MB were identified; by WHO definition, most of these resided within the classic category (N = 19), while one was LCA. 13 of 20 B-MB displayed 'scattered" nodules; by molecular subtype, these included eight group 4, four group 3 and one WNT tumors. Seven of the 20 B-MB exhibited "frequent" nodules; by molecular subtype, these included six group 4 and one group 3 tumors. Statistical analysis confirmed this non random distribution of B-MB across molecular subtypes.

Conclusion: Our data confirm the work of Ellison et al. that suggested B-MB is genetically different than DN-MB. In particular, B-MB resides in the non-WNT/SHH molecular category, but especially amongst group 4 when nodularity is "frequent".

5. Automated analysis of 1p/19q status by FISH in oligodendroglial tumours.

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Automated analysis of 1p and 19q status in oligodendroglial tumors by fluorescence in-situ hybridization (FISH) can be achieved by image-analysis software present in the majority of institutions using the FISH technique. Despite the widespread availability of this software, there are no specific guidelines in the literature on how to use it.

We studied which green/red (G/R) probe signal combinations are predictive of 1p/19q co-deletion in a retrospective series of 53 oligodendroglial tumours and defined a new algorithm with a reduced sequence of combinations compared to previous studies. This algorithm was then tested and refined on a prospective series of 45 oligodendroglial tumours. The new algorithm scores 24 G/R combinations, which represent less than 50 % of the total observed combinations in our series. This algorithm excludes some previously described combinations and redefines the place of others. G/R combinations of 5/2, 6/2 and 6/3 associate with deletion status combinations, combinations of 1/2 associate with normal chromosome status, and combinations of 3/3 and 4/4 associate with imbalanced chromosome status.

The new algorithm when applied to the combination and ratio methods of signal probe analysis gives a high concordance between manual and automated analysis on examination of 100 tumour cells (91% concordance for 1p and 89% concordance for 19q) and total concordance on examination of 200 tumour cells. This highlights the value of automated analysis to identify cases with imbalanced chromosome status, in which a larger number of tumour cells should be study by manual analysis. Our algorithm can be easily programmed on all existing FISH analysis software platforms and should facilitate multicentric evaluation and standardization of 1p/19q assessment in gliomas.

6. Surfen, a proteoglycan antagonist, reduces lysolecithin-induced demyelination with related effects on macrophage function

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Proteoglycans are components of the extracellular matrix and have roles in brain development and responses to injury. Connective tissue components are known to be major inhibitors of remyelination in mouse models of demyelination and are found at the border of active demyelinating lesions in Multiple Sclerosis. Surfen (bis 2-methyl, 4- amino, 6-quinolyl amide) is a small molecule antagonist previously shown to bind preferentially to heparan sulfate and related proteoglycans.

We have previously reported that surfen reduces T cell proliferation *in vivo* and *in vitro*. Here we report the effects of surfen on an *in vivo* model of demyelination and its effects on macrophage function *in vitro*. Demyelination was induced by injecting the detergent lysolecithin into the spinal cord dorsal columns of adult C57Bl/6 mice. Relative to vehicle treated mice, co-injection of surfen (100 μM) with lysolecithin reduced total lesion area seven days post-injection. Because macrophages dominate these lesions and influence remyelination, murine bone marrow derived macrophages were assessed using assays of chemotaxis and phagocytosis. Macrophages chemotaxis was increased in response to surfen (10 μM) relative to vehicle by approximately 15% ($p < 0.05$). Phagocytosis of E. coli was not affected by surfen.

These effects of surfen on experimental demyelination and macrophage function suggest that proteoglycan binding may promote aspects of myelin repair relevant to Multiple Sclerosis.

7. The pathogenesis of Friedreich cardiomyopathy: myocarditis

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Frataxin deficiency causes the complex neurological and cardiac phenotype of Friedreich ataxia (FRDA). The most common cause of death is cardiomyopathy. The results presented here are based on a systematic study of fixed and frozen archival heart specimens and include measurement of cardiomyocyte hypertrophy, frataxin assay, X-ray fluorescence (XRF) of iron (Fe) and zinc (Zn), inductively-coupled plasma optical emission spectrometry of these metals in digests of left ventricular wall (LVW), right ventricular wall (RVW), and ventricular septum (VS), Fe histochemistry, and immunohistochemistry and double-label immunofluorescence microscopy of cytosolic and mitochondrial ferritins, and of the inflammatory markers CD68