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Review

Pediatric brain tumors: Update of proteome-based studies

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ABSTRACT

Pediatric brain tumors (PBTs) are the most common solid malignancies in childhood and continue to pose a serious burden to modern societies. Existing treatments impose debilitating effects on the developing child, highlighting the need for molecularly targeted treatments with reduced toxicity, as well as the necessity of markers that reliably assess efficacy of, and tumor response to targeted-therapies of PBTs. On this regard advances in technologies of protein identification and quantification, the large-scale, high-throughput investigation of the proteome, as well the newly-emerging field of "proteogenomics" aim to further our knowledge towards understanding the molecular pathophysiology of PBTs. This mini review article presents all updates on knowledge produced and published during the last years on PBT research derived from "omics" technologies, mainly involving protein research and proteomics.

Pediatric brain tumors (PBTs) are the most common solid malignancies in childhood and the most common cause of cancer-related mortality and morbidity in this age group. Diagnosis and treatment of these tumors have undergone significant improvements over the last decades, starting with implementation of imaging techniques like computed tomography (CT) and magnetic resonance imaging (MRI). Neuropathology has, since, moved from an essentially morphology-based approach to more sophisticated discipline applying molecular biological techniques that provide new insight in this group of tumors, by allowing identification of molecular targets [1].

The need of markers that reliably assess efficacy of, and tumor response to, specific biologic-targeted therapies (*i.e.* small-molecule inhibitors and anti-angiogenic agents), is greater than ever. These marker/molecules should reliably detect minimal residual disease, aid in the rational selection of new agents for investigation in clinical trials and new targets for novel drug design and therapeutic strategies. Lastly, markers that better predict response to specific targeted-therapies augmenting the ability of neuroimaging studies and differentiating tumors from post-surgical or radiation-induced effects are of dire need.

Some of the needs mentioned above are being addressed through advances in technologies of protein identification and quantification, that make possible the large-scale, high-throughput investigation of the proteome. Furthermore, it is nowadays quite common to see genomics data informing proteomics data analysis or *vice versa*; the newly emerging field of "proteogenomics".

This mini review article aims at updating knowledge produced and published during the last years since our last review article [1] on PBT research based on "omics" technologies, mainly involving protein research and proteomics.

1. Available tissues for PBT research

Given the sensitivity of currently existing analytical methods, primary tumors are amenable to direct molecular analysis. Clinical specimens have been used by researchers for the study of mutations, copy number variations, differential transcript and proteome profiles. However, the availability of adequate amount of material from PBTs and the extent of heterogeneity in specimens pose serious challenges for any comprehensive study and clinical correlations of these tumors. Furthermore, direct molecular analysis between tumor and the non-tumor tissue from the same patient is also difficult on account of tumor boundaries. Tissue collected from temporal lobe epilepsy surgeries are usually used instead as experimental controls, but they most often come from different subjects than tumor donors. Despite these limitations, primary brain tumors are important for analysis and have been used with reasonable success in PBT research [2].

1.1. Cerebrospinal fluid

Cerebrospinal fluid (CSF)1 surrounds and supports the central nervous system (CNS), including the ventricles and subarachnoid space [3]. About 80% of the total protein amount in CSF derives from size-dependent filtration of blood across the blood-brain barrier (BBB), and the rest originate from drainage of interstitial fluid from the CNS [4,5].

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Because CSF is in direct contact with the CNS, it should be a promising source for finding biomarkers for diseases in the CNS [6].

In their latest study, Spreafico et al. sought to characterize the CSF proteome of patients with CSF tumors to identify biomarkers predictive of metastatic spread [7].

CSF samples from children with brain tumors and controls (extra-CNS non-Hodgkin's lymphoma) were processed reverse-phase liquid chromatography/electrospray tandem mass spectrometry (LC-MS/MS). Six proteins (type 1 collagen, insulin-like growth factor binding protein 4, procollagen C-endopeptidase enhancer 1, glial cell-line derived neurotrophic factor receptor α2, inter-alpha-trypsin inhibitor heavy chain 4, neural proliferation and differentiation control protein-1) revealed the ability to discriminate metastatic cases from controls. Their analysis also included validation of results by reverse phase protein array (RPPA), western blot and ELISA.

Since CSF biomarker discovery, in general, suffers important physiological and technical challenges, including: low protein levels and the presence of highly abundant proteins masking less abundant ones, the authors firstly relied on initial non-targeted discovery phase, selecting 26 top proteins. These initial molecules underwent further quantitative validation, and were scored regarding their ability to distinguish CSF samples between metastatic cases and controls. The authors ultimately highlighted six proteins as potential biomarkers of meningeal spread. Most important aspect of the study was the coupling of MS analysis with a core-shell nanoparticle capture technique, enabling for identification of a large number of low-weight and low-abundant proteins, not previously described in the CSF.

1.2. Biopsy tissue samples

A number of medium-scale expression studies characterizing the levels of mRNA and proteins expressed in normal and cancerous tissue have been undertaken with the goal of identifying unique expression signatures associated with cancer phenotype [8,9]. Although a wealth of information exists on the expression signature of mRNAs expressed by a specific type of cancer, little data are available about the protein expression signatures that exist in normal and cancerous prostate tissue.

Massimi et al. investigated the molecular characteristics of Adamantinomatous craniopharyngioma (ACP), a tumor entity often burdened by a poor prognosis in children as far as risk of recurrence and quality of life are concerned, aiming at finding new therapeutic options [10]. This was an addition to an already published study by the same group covering the basic proteomic traits of ACP [11]. In both studies “bottom-up” and “top-down” LC-MS/MS approaches were utilized in the study. The bottom-up approach pointed out several proteins of inflammation (namely, α2-HS-glycoprotein, α1-antichymotrypsin and apolipoproteins) as involved in genesis and growth of the cystic component of ACP. The top-down strategy analyzed proteins and peptides in the intact state, thus being suitable for identification of peptides and low-molecular weight proteins and characterization of their possible isoforms and post-translational modifications. The top-down approach disclosed the presence of the thymosin β family. Thymosin β4 is involved in the cytoskeleton organization and migration of several tumors, could play a role in the progression of ACP. Finally, PA was utilized to investigate alterations in cyst fluid character after treatment with interferon-α. The analyzed samples showed a progressive reduction of the levels of α-defensins (proteins involved in the inflammatory-mediated response) after the intracystic injection of interferon-α, thus reinforcing the hypothesis that inflammation contributes to ACP cyst pathogenesis.

It is important that, through this analysis, the proteomic characterization of ACP cyst fluid led to identification of proteins strongly connected with mineralization process, lipid transport, and the inflammatory response, that are in total accordance with ACP cyst fluid composition (lipids, granules of cholesterol, flecks of calcium). It is evident form their results that for the specific malignancy, as well as a number of other cases, inflammation plays a crucial role in the development of cystic ACP (cyst formation and growth). Furthermore, key points of their study, including a-defensins and b-thymosins, deserve greater attention as potential molecules for targeted therapy, since they seem to be centrally involved in ACP tumor growth, progression and infiltration.

In 2014, Inserra et al. applied LC coupled to LTQ-Orbitrap analysis for a proteomic study of pediatric pilocytic astrocytoma brain tumor intracystic fluid by an integrated top-down/bottom-up platform [12]. By incorporating both approaches the authors aimed at achieving a wide characterization of the protein and peptide content of the tumor fluid. The top-down approach allowed identification of several proteins and peptides involved in different biological activities together with the characterization of interesting proteoforms such as fibrinopeptide A and its truncated form, fibrinopeptide B, complement C3f fragments, β-thymosin peptides, ubiquitin, several apolipoproteins belonging to A and C families, apolipoprotein J and D, and cystatin C. Of particular importance are their findings of a N-terminal truncated cystatin C proteoform, involved in immune response mechanism modulations and the identification of oxidized and glycosylated apolipoproteins including disulfide bridge dimeric forms. Their bottom-up approach confirmed some of the experimental data findings together with adding the characterization of high-molecular-mass proteins in the samples.

It is important to note that their top-down approach highlighted proteins' post-translational modifications, thus advancing comprehension of molecules with importance in disease onset and development, such as truncated, oxidized, and glycosylated proteoforms, while allowing for recognition of high-molecular-mass proteins thus widening proteome characterization. Key results of this approach include several proteins belonging to the apolipoprotein family presenting different post-translational modifications, including the presence of dimeric forms, and the characterization of truncated cystatin C, missing the first four N-terminal amino acid residues, and reported to strongly modulate the human immune defense mechanisms

Our group recently reported on a proteomics-based analysis of childhood medulloblastoma tissues. Our experiments were performed on tissue specimen collected at surgery. With 17p loss being the commonest chromosomal aberrance observed in our analyzed sample set, array-CGH were employed to first distinguish for 17p-positive cases, which were further processed. 2-DE coupled to MS identification, fully-exposed the MBL-specific protein profile. Protein profiles of malignant tissues (surgically removed specimens) were compared against profiles of “normal” cerebral (non-malignant parts of surgical specimens) tissues and quantitative protein differences also determined. Bioinformatics, functional and database analyses, characterization and classification and sub-network profiling, generated information on MBL protein interactions. Our results confirmed dominance of the PI3K/mTOR signaling network for the specific childhood brain malignancy. Furthermore, 380 single-gene products were uniquely associated with the MBL phenotype. Quantitative assessment revealed 200 proteins to be up-regulated and 150 down-regulated, in MBL tissues compared to normal cerebral controls. IGF2, PI3R5, Raf-1, Rictor, MAPK1, S6K1, 4EBP1 and ELF4A, as part of the IGF system (implicating PI3K/mTOR) were deemed to be de-regulated. By the specific approach and by deciphering of the MBL proteome we showed that mTOR/PI3K signaling de-regulation is of dominant importance in 17p MBL cases [13].

1.3. Tumor-derived cell lines

Tumor-derived cell lines constitute a complementary alternative to study molecular aspects relevant to the tumors. Several studies have used cell lines for targeted functional studies as well as high-throughput molecular analysis under defined con- ditions to understand molecular mechanisms underlying PBTs mechanisms of tumorigenesis [14–16]. Cell line studies are also useful for identifying tumor-associated molecules with secretory potential from their conditioned medium. In the