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Research Paper

Circulating Micrornas Predict Survival of Patients with Tumors of Glial Origin

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ABSTRACT

The World Health Organization has recently introduced molecular prognostic-diagnostic biomarkers in the classification of Central Nervous System (CNS) tumors. In order to characterize subclasses of tumors that cannot find a precise location in the current classification, and, or cannot be tested because of scant material, it is important to find new molecular biomarkers in tissue and, or biological fluid samples. In this study, we identified serum microRNAs that could serve as biomarkers for the diagnosis and prognosis of patients with tumors of glial origin. We retrospectively analyzed microRNA expression in the serum extracellular vesicles of patients with tumors of glial origin. Extracellular vesicles RNA was analyzed by Nanostring. qRT-PCR confirmed 6 overexpressed microRNAs: hsa-miR-4443, hsa-miR-422a, hsa-miR-494-3p, hsa-miR-502-5p, hsa-miR-520f-3p, and hsa-miR-549a. Hsa-miR-4443 was the only microRNA that showed significant differences in most comparisons. In situ hybridization (ISH), confirmed that our signature was mostly expressed in cancer cells.
Importantly, hsa-miR-549a and hsa-miR-502-5p expression predicted prognosis in patients with tumors of glial origin. Although more studies are needed, we demonstrated that serum vesicles microRNA profiles are promising diagnostic and prognostic molecular biomarkers that will find an actual application in the clinical practice of CNS tumors.

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1. Introduction

In 2016, the World Health Organization (WHO) published a new classification for Central Nervous System (CNS) tumors that, beside deleting and adding histological subgroups, introduced, for the first time, molecular biomarkers that define new prognostic groups (Louis et al., 2016). In attempt to merge the different clinical information contributed by previous classifications, the International Society of Neuropathology (ISN) proposed a "layered" diagnostic path, that includes the histologic classification (layer 2), WHO grading (layer 3), molecular information (layer 4) and an "integrated diagnosis" (layer 1) (Louis et al., 2014).However, because of their extensive biological variability some gliomas cannot be molecularly classified (Masui et al., 2016). Furthermore, a group of glial tumors has not yet found a precise molecular classification: the "Not Otherwise Specified" (NOS) cases include those tumors for which testing cannot be performed due to limited tissue availability, low number of neoplastic cells, or other causes (Louis et al., 2016). Clearly, patients with a non-classified biological variant of gliomas and/or a "NOS", will not be able to benefit of the molecularly targeted treatment. On the other hand, imaging studies cannot microscopically define clear margin in malignant lesions before and after surgery. Medications affecting Blood Brain Barrier (BBB) permeability, can produce images with falsely reduced tumor burden ("pseudo-response") (Holdhoff et al., 2013). Treatment of high grade gliomas with radiation and temodar can lead to "pseudo-progression", causing unnecessary surgery or chemotherapy termination (Santangelo et al., 2017). It is extremely important to find molecular biomarkers aiding in the diagnosis and classification of these tumors on tissue samples and, more importantly, on biological fluids. MicroRNAs are non-

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coding small RNAs of few nucleotides (Li et al., 2012; Ostrom et al., 2015; Peterson et al., 2015; Skog et al., 2008; Van Deun and Hendrix, 2017) in length that modulate gene expression by regulating mRNA translation/degradation, playing key roles in physiologic and pathologic processes, including cancer (Drusco and Croce, 2017). Moreover, microRNA profiles can classify human cancer, rendering these small molecules suitable biomarkers to classify cancers for diagnostic, prognostic and therapeutic purposes (Lu et al., 2005). MicroRNAs are expressed in all tissues and biological fluids (Drusco et al., 2015; Mitchell et al., 2008; Russo et al., 2017), where they are released either freely as chemically modified molecules, or associated with proteins, or within extracellular vesicles (EVs) (Raposo and Stoorvogel, 2013). Secreted by all type of cells, including cancer cells, EVs are a system of intercellular communication through which proteins and nucleic acids are packed within small membrane spherules and shipped at distant sites (Taylor and Gercel-Taylor, 2008). Thus, microRNA profiling in EVs of patients' biological fluid, may lead to the identification of new diagnostic and prognostic biomarkers. In this study, we identified a microRNA signature of serum EVs of patients with tumors of glial origin that was not only able to differentiate between glioma and normal patients, but also to predict survival.

2. Materials and Methods

2.1. Samples

Serum samples from 9 patients with Oligodendroglioma, 9 patients with Astrocytoma and 10 patients with Glioblastoma were provided by UCSF neurosurgery department (Genomic Shared Resources Core is NIH subsidized Shared Resources CCSG:P30CA016058), while 8 normal controls were provided by Ohio State University, Columbus, OH (Protocol: 2005C0014) (Fig. 1). In situ hybridization FFPE sections of 12 oligodendrogliomas, 10 astrocytomas, 13 glioblastoma samples and 12 non-neoplastic perilesional grey matter specimens were provided by the Regina Elena Institute, Dept. of Pathology, Rome (Protocol: 825/16).Collection of tissue and serum samples has been conducted according to the standards established by the Declaration of Helsinki, and informed consent was obtained according to the approved protocols mentioned above.

2.2. Extracellular Vesicles RNA Extraction

In order to avoid any cellular contamination samples were centrifuged 3 times at 3500 rpm for 20 min. After each centrifugation, supernatant was transferred to a new tube. Extracellular vesicles were extracted from 250μl of serum using Exoquick plus (System Bioscience cat#EXOQ5TM-1) and following manufacturer's instructions. The exosomal pellet was resuspended in 1.5 ml of TRIReagent (SIGMA-ALDRICH cat#T9424-200 ml), and 2 μl of 33.3 attoMole solution of spike-ins (cel-miR-248 and ath-miR159a) were added to each sample. The RNA was then extracted using the NORGEN RNA Clean-up and Concentration kit (cat#23600) and RNA was isolated following manufacturer's instructions.Furthermore, samples were concentrated and cleaned up from any chemical residues by centrifugation through Amicon ultra 0·5 centrifuge filters (SIGMA ALDRICH cat#Z740169-96EA), and then speed-vacued to 10 μl of volume.

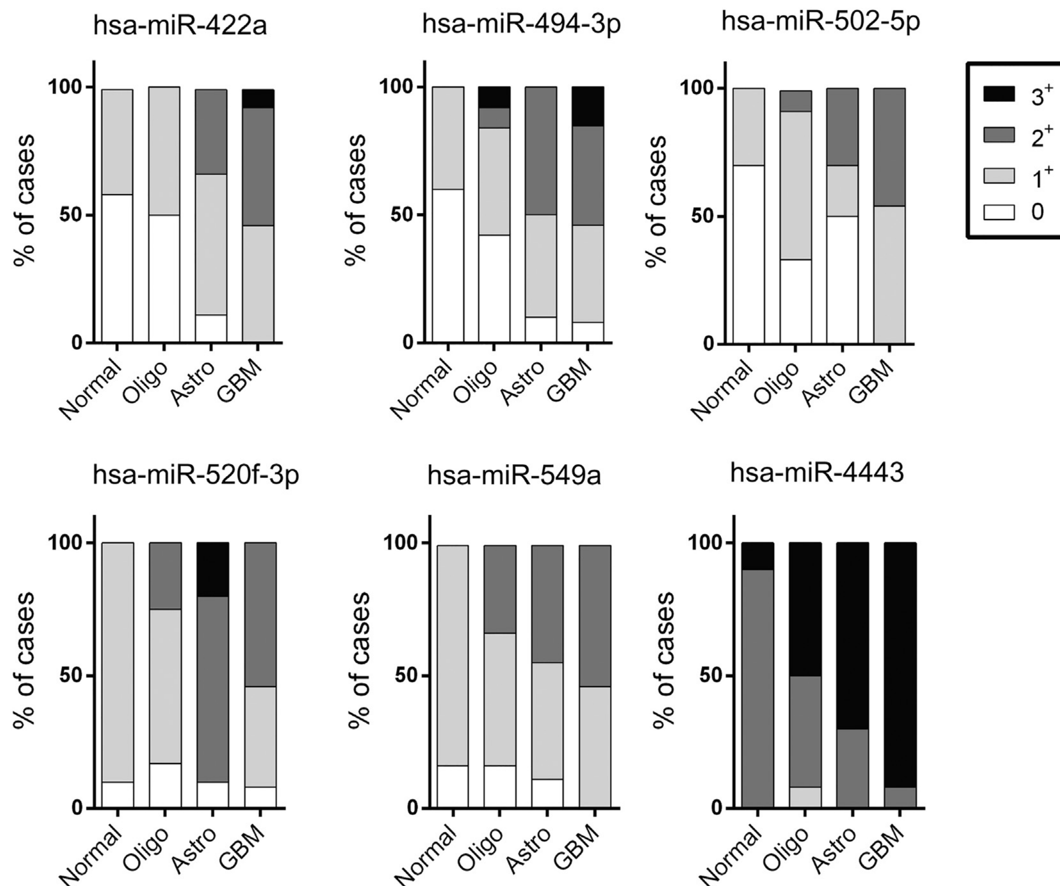

Fig. 1. In situ hybridization histograms. ISH was performed on 12 Normal CTRL (perilesional grey matter), 12 Grade II oligodendrogliomas, 10 Grade II astrocytomas and 13 glioblastomas. The intensity of expression of each microRNA of the signature was scored in each group of Glioma with numbers going from 0, in case of absent expression, to 3+ for maximal expression.

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