Experimental Cell Research 339 (2015) 280–288

Contents lists available at ScienceDirect

Experimental Cell Research

journal homepage: www.elsevier.com/locate/yexcr

Research Article

High levels of WNT-5A in human glioma correlate with increased presence of tumor-associated microglia/monocytes

Jacomijn P. Dijksterhuis [a], Elisa Arthofer [a], Voichita D. Marinescu [b], Sven Nelander [b], Mathias Uhlén [c], Frederik Pontén [b], Jan Mulder [d], Gunnar Schulte [a,e,*]

[a] Section of Receptor Biology & Signaling, Deptartment Physiology & Pharmacology, Karolinska Institutet, S17177, Stockholm, Sweden
[b] Department of Immunology, Genetics and Pathology (IGP), Science for Life Laboratory, Uppsala University, SE-751 85 Uppsala, Sweden
[c] Science for Life Laboratory, KTH-Royal Institute of Technology, SE-171 21 Stockholm, Sweden
[d] Science for Life Laboratory, Department of Neuroscience, Karolinska Institutet, S-17177 Stockholm, Sweden
[e] Faculty of Science, Institute of Experimental Biology, Masaryk University, 611 37 Brno, Czech Republic

ARTICLE INFO

Article history:
Received 17 July 2015
Received in revised form
16 October 2015
Accepted 23 October 2015
Available online 25 October 2015

Keywords:
WNT-5A
Microglia
Glioma
Major histocompatibility complex II
Tumor microenvironment

ABSTRACT

Malignant gliomas are among the most severe types of cancer, and the most common primary brain tumors. Treatment options are limited and the prognosis is poor. WNT-5A, a member of the WNT family of lipoglycoproteins, plays a role in oncogenesis and tumor progression in various cancers, whereas the role of WNT-5A in glioma remains obscure. Based on the role of WNT-5A as an oncogene, its potential to regulate microglia cells and the glioma-promoting capacities of microglia cells, we hypothesize that WNT-5A has a role in regulation of immune functions in glioma. We investigated WNT-5A expression by *in silico* analysis of the cancer genome atlas (TCGA) transcript profiling of human glioblastoma samples and immunohistochemistry experiments of human glioma tissue microarrays (TMA). Our results reveal higher WNT-5A protein levels and mRNA expression in a subgroup of gliomas (WNT-5A^{high}) compared to non-malignant control brain tissue. Furthermore, we show a significant correlation between WNT-5A in the tumor and presence of major histocompatibility complex Class II-positive microglia/monocytes. Our data pinpoint a positive correlation between WNT-5A and a proinflammatory signature in glioma. We identify increased presence of microglia/monocytes as an important aspect in the inflammatory transformation suggesting a novel role for WNT-5A in human glioma.

1. Introduction

WNT-5A, a secreted lipoglycoprotein of the Wingless/Int1 (WNT)[#] family of proteins, serves as ligand for Frizzleds (FZD), which belong to the Class Frizzled of G protein-coupled receptors [1,2]. The highly conserved WNT/FZD signaling system is known to be essential for embryonic development, and is involved in tissue homeostasis and immunological processes. Deregulation of the pathway can lead to various diseases including cancer and neurological disorders [1,3,4]. The role of WNT-5A in cancer is dual and varies between cancer types. For instance, while WNT-5A exerts tumor-suppressive functions in thyroid carcinoma [5] and breast cancer [6], it can also promote invasion and migration in gastric cancer cells [7] and melanoma [8].

Due to the variability, invasiveness and aggressiveness of gliomas, success rates of therapies like surgical resection and chemo- or radiation therapy are low, resulting in poor prognosis for the patient [9]. Gliomas comprise heterogeneous cell populations including tumor (initiating) cells and tumor-associated cells. Tumor associated cells, including microglia, astrocytes and macrophages play a significant role in tumor aggressiveness, progression and influence patient survival [10]. The regulatory role and molecular mechanisms underlying WNT-5A signaling in glioma development and progression is unknown and remains to be elucidated. Previous studies indicated a role of WNT-5A in glioma cell proliferation and invasiveness [11,12].

Our work recently revealed a novel role for WNT-5A in the immunological regulation of microglia, manifested by induction of proliferation, expression of proinflammatory cytokines and

Abbreviations: FZD, Frizzled; GFAP, glial fibrillary acidic protein; GBM, glioblastoma multiforme; HLA, human leukocyte antigens; IBA-1, ionized calcium-binding adapter molecule 1; MHCII, major histocompatibility complex II; TCGA, The Cancer Genome Atlas; TMA, tissue microarray; WNT, Wingless/Int-1 family proteins

* Corresponding author at: Section of Receptor Biology & Signaling, Deptartment Physiology & Pharmacology, Karolinska Institutet, S17177 Stockholm, Sweden. Fax: +46 8 341280.
E-mail address: gunnar.schulte@ki.se (G. Schulte).

http://dx.doi.org/10.1016/j.yexcr.2015.10.022
0014-4827/

chemokines as well as increased expression of matrix metalloproteases supporting WNT-5A-induced microglia invasion in vitro [13]. In the context of glioma biology, this activity profile is of particular interest since it is shown repeatedly that microglia support glioma invasion in the brain, rendering tumors more aggressive and diffuse, and thereby even more difficult to treat [14,15]. In fact, a high number of microglia in the tumor microenvironment positively correlates with invasiveness and thereby aggressiveness of the cancer [16–18]. Interestingly, Pukrop et al. showed that WNT-5A signaling through microglia assists breast tumor metastases in the brain, rendering our hypothesis valid for another tumor type showing high WNT-5A levels [19,20].

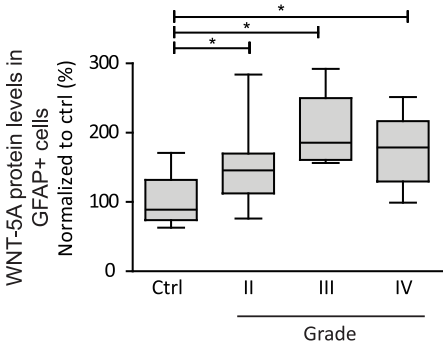
Here we examine the expression levels of WNT-5A in patient-derived gliomas and investigate the association of WNT-5A and microglia responses in the tumor microenvironment. Based on an *in silico* analysis of the The Cancer Genome Atlas (TCGA) glioblastoma multiforme (GBM) sample set and immunohistochemistry experiments on human glioma TMAs we identified high expression of WNT-5A in a subset of human gliomas compared to control tissue. Furthermore, by using the TCGA GBM sample set we found a strong positive correlation between WNT-5A expression and a strong proinflammatory signature based on the presence of microglia/monocytes. Thus, we provide a novel link between WNT-5A levels and glioma-associated immune cells in human glioma extending recently published *in vitro* findings on the WNT-5A-induced increase in microglia proliferation and invasion.

A

	Fold Change	*P* value	Reporter ID
WNT-1	-1.155	0.998	208570_at
WNT-2	-1.786	1.000	07-116727251
WNT-2B	-1.221	0.997	206458_s_at
WNT-3	-1.090	0.967	221455_s_at
WNT-4	-1.113	0.999	208606_s_at
WNT-5A	4.068	7.64 E-13	205990_s_at
WNT-5A	4.336	2.58 E-14	213425_at
WNT-5B	-1.286	0.995	221029_s_at
WNT-6	-1.076	0.872	221608_at
WNT-7A	-1.487	0.999	210248_at
WNT-7B	-1.642	1.000	217681_at
WNT-8B	-1.250	1.000	207612_at
WNT-10B	-7.016	1.000	206213_at
WNT-11	-1.168	0.996	206737_at
WNT16	1.096	0.146	221113_s_at

General information	**Filters:**
Measurement: mRNA	Gene: WNTx
Human Genome U133A Array	Analysis Type: Cancer vs. Normal Analysis
Samples Brain: 10	Cancer Type: Brain Glioblastoma
Samples Brain Glioblastoma: 542	Dataset: TCGA Brain

C



D

		Patient with	
		Low WNT-5A (%)	High WNT-5A (%)
Gender	Male	55.63	60.90
	Female	44.37	39.10
Subtype	Classical	49.62	9.77
	Mesenchymal	20.30	45.11
	Proneural	18.04	28.57
	Neural	12.03	16.54

B

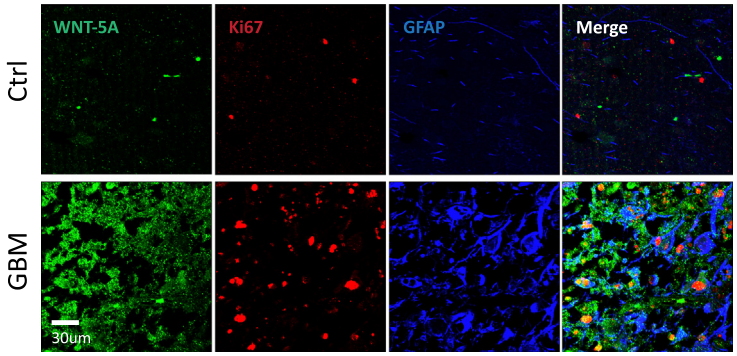


Fig. 1. WNT-5A is overexpressed in human glioma. (A) Visualized WNT mRNA expressions between non-malignant control brain tissue ($n=10$) and brain GBM ($n=542$) and their associated probe ID. Analysis was performed with the TCGA GBM data set using Oncomine bioinformatics software. WNT-5A mRNA expression is 4.068 fold increased ($p=7.64\ E^{-13}$) between control and GBM samples. The probe ID for WNT5A used was 205990_s_at. WNT-5A mRNA (FC – fold change) with probe ID 213425_at was 4.336 ($p=2.58\ E^{-14}$). (B) Confocal micrographs of human diffuse astrocytoma showing grade II and control brain tissue from a tissue microarray. Ki67 and GFAP label proliferating cells and astrocytes, respectively. (C) Quantification of WNT-5A protein expression from the TMA immunohistochemistry in $GFAP^{+}$ cells in astrocytoma grade II, anaplastic astrocytoma grade III and GBM grade IV normalized to control brain tissue. The middle line represents the median. Data were analyzed with unpaired Student's *t* test ($^{*}p<0.05$). (D) WNT-5A mRNA expression distribution among the TCGA glioblastoma sample set.