Aus dem Max Delbrück Centrum für Molekulare Medizin

DISSERTATION

Investigating the role of Toll-like receptors in the Glioma Microenvironment

zur Erlangung des akademischen Grades
Medical Doctor - Doctor of Philosophy (MD/PhD)
im Rahmen des
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Table of Contents

Abstract German

Periphere Makrophagen und residente Mikroglia stellen die dominante Glioblastom infiltrierende Zellpopulation dar. Das Glioblastom induziert einen immunsupprimierten und Tumor fördernden Phänotyp in den Glioblastom assoziierten Mikroglia und Makrophagen. Eine Subpopulation der Glioblastom-Zellen hat Stammzelleigenschaften wie: Selbsterneuerung, Multipotenz und agieren als Glioblastom-Stammzellen. In der aktuellen Studie untersuchten wir die Interaktion zwischen Glioblastom-Stammzellen und Glioblastom assozierten Mikroglia und Makrophagen. Unter Nutzung des Tumorstammzellmarkers CD133 haben wir die Maus Glioblastom-Zelllinie GL261 angereichert bzw. befreit von Glioblastom-Stammzellen mittels Durchflusszytometrie. 100 $CD133^{+}$ Glioblastom-Stammzellen besaßen die Fähigkeit über die selbe Zeitspanne Tumoren von vergleichbarer Größe zu initiieren wie 10000 $CD133^{-}$ GL261-Zellen. In der IL-$6^{-/-}$ Maus waren nur Tumore aus $CD133^{+}$ Zellen kleiner im Vergleich zur Wildtyp Maus. Nach Stimulation von primär kultivierten Mikroglia mit konditioniertem Medium von CD133 angereicherten GL261-Zellen konstatierten wir eine Hochregulierung der mikroglialen IL-6 Sekretion, wobei im Vergleich konditioniertes Medium von CD133 befreiten GL261-Zellen die Hochregulierung nicht auslöste. Diese Zytokinsekretions-Hochregulierung galt selektiv für IL-6 im Vergleich zu einem Panel an anderen Zytokinen (wie zum Beispiel TNF-α oder IL-4). Des Weiteren konnten wir feststellen, dass diese Hochregulation von Toll-like Rezeptor 4 abhängig war. Toll-like Rezeptor 4 ist ein zu der Gruppe der Pattern Recognition Rezeptoren zählende Struktur, welche die Sekretion von pro-inflammatorischen Zytokinen auslösen kann. Die Schlussfolgerung der TLR4-Abhängigkeit konnten wir ziehen, weil der Effekt der IL-6 Sekretions-Hochregulierung in der $TLR4^{-/-}$ ausgelöscht war, nicht aber in Linien mit Knockout für andere Toll-like Rezeptoren. Unsere Ergebnisse zeigen auf, dass Glioblastom-Stammzellen spezifisch eine mikrogliale IL-6 Sekretion mittels TLR4 Signalweg auslösen und IL-6 wiederum Tumorwachstum fördernd wirkt durch Unterstützung der Glioblastom-Stammzellen. Unter Nutzung von humanem Glioblastomgewebe konnten wir unsere Entdeckung verifizieren, dass Glioblastom assoziierte Mikroglia und Makrophagen die Hauptquelle für IL-6 im Tumorkontext sind.

Abstract English

Peripheral macrophages and resident microglia constitute the dominant glioma-infiltrating cells. The tumor induces an immunosuppressive and tumor supportive phenotype in these glioma associated microglia/brain macrophages (GAMs). A subpopulation of glioma cells has stem cell properties such as self-renewal, multipotency and act as glioma stem cells (GSCs). In the present study we explored the interaction between GSCs and GAMs. Using CD133 as a marker of stemness, we either enriched for or deprived the mouse glioma cell line GL261 of GSCs by FACS. Over the same period of time, 100 $CD133^{+}$ GSCs had the capacity to form a tumor of comparable size to the ones formed by 10000 $CD133^{-}$ GL261 cells. In IL-$6^{-/-}$ mice, only tumors formed by $CD133^{+}$ cells were smaller when compared to wild-type. After stimulation of primary cultured microglia with conditioned medium from CD133 enriched GL261 glioma cells, we observed an upregulation in microglial IL-6 secretion while medium from CD133 deprived gliomas did not trigger this release. This upregulation was selective for IL-6 as compared to a battery of other cytokines (e. g. TNF-α or IL-4). This upregulation depended on Toll-like receptor (TLR) 4, a pattern recognition receptor which can trigger pro-inflammatory cytokine release, since the effect was abolished in the $TLR4^{-/-}$ mouse, but not in other strains deficient for other TLRs. Our results show that GSCs, but not the bulk glioma cells, initiate microglial IL-6 secretion via TLR4 signaling and that IL-6 regulates glioma growth by supporting GSCs. Using human glioma tissue we could confirm the finding that GAMs are the major source of IL-6 in the tumor context.

Affidavit

“I, Omar Dzaye, certify under penalty of perjury by my own signature that I have submitted the thesis on the topic “Investigating the role of Toll-like receptors in the Glioma microenvironment”. I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The sections on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) correspond to the URM (s.o) and are answered by me. My interest in any publications to this dissertation corresponds to those that are specified in the following joint declaration with the responsible person and supervisor. All publications resulting from this thesis and which I am author correspond to the URM (see above) and I am solely responsible.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

Date Signature

Detailed declaration of contribution

Publication :

Omar Dildar a Dzaye*, Feng Hu*, Katja Derkow, Verena Haage, Philipp Euskirchen, Christoph Harms, Seija Lehnardt, Michael Synowitz, Susanne A. Wolf and Helmut Kettenmann

„Glioma stem cells but not bulk glioma cells upregulate IL-6 secretion in microglia/brain macrophages via Toll-like receptor 4 signaling.“

*Co-first author, Journal of Neuropathology and Experimental Neurology (2016): accepted and in press

Contribution:

Conceived the study together with Helmut Kettenmann and Susanne Wolf. Performed the majority of the in vitro, ex vivo and in vivo experiments (Figures 1,3,4 and 5; Supplementary figures 1,2,3 and 4). Responsible for cell culture of the following cell lines: GL261, C6, U87 and NCH421K. Participated in TLRs KO primary microglia culture preparation. Performed magnetic-activated cell sorting (MACS) and fluorescence-activated cell sorting (FACS) isolation of glioma cancer stem cells. Checked the purity of stem cells every second day by FACS analysis. Prepared glioma cell-conditioned medium for the in vitro experiments. Measured the protein concentration of all cell-conditioned media. Performed ELISA and Multiple Analyte Detection analysis. Was responsible for the TLR4 antibody treatments. Designed all primers and performed qPCR experiments togehter with Verena Haage. Contributed to tumor inoculation together with Feng Hu. Was responsible for the daily care of all in vivo experiments. Organized the TLRs KO and IL-6 KO animals. Performed immunofluorescent stainings and processed the images. Quantified tumor volume in brain slices of glioma–bearing mice according to the Cavalieri principle. Performed statistical analysis together with Feng Hu and Susanne Wolf. Wrote the paper together with Feng Hu, Susanne Wolf and Helmut Kettenmann.

Signature, date and stamp of the supervising University teacher

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Signature of the doctoral candidate

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[1 | 2 | 3 | 4]

Page 1 of 4

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☐	20	ARCH PATHOL LAB MED	0003-9985	9276	2.838	2.902	0.677	198	9.1	0.01373	0.883

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Glioma stem cells but not bulk glioma cells upregulate IL-6 secretion in microglia/brain macrophages via Toll-like receptor 4 signaling

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Curriculum Vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Complete publication list

Omar Dildar a Dzaye*, Feng Hu*, Katja Derkow, Philipp Euskirchen, Christoph Harms, Seija Lehnrdt, Michael Synowitz, Susanne A. Wolf and Helmut Kettenmann. "Glioma-initiating cells but not bulk glioma cells upregulate IL-6 secretion in microglia/brain macrophages via Toll-like receptor 4 signaling"
*Co-first author, Journal of Neuropathology and Experimental Neurology (**2016**): 27030742 (IF=3,797)

Feng Hu*, **Omar Dildar a Dzaye***, Alexander Hahn, Yong Yu, Adrian Kamil Kaczmarek, Carmela Ricciardelli, Michael Synowitz, Susanne A. Wolf and Helmut Kettenmann. "Versican released from glioma converts microglia/brain macrophages into a pro-tumorigenic phenotype via Toll-like receptor 2 signaling."
*Co-first author, Neuro-Oncology (**2015**): 25452390 (IF=6,776)

Benjamin Förstera*, **Omar Dildar a Dzaye***, Aline Winkelmann, Marcus Semtner, Bruno Benedetti, Ralf P. Friedrich, Darko S. Markovic, Carola Bernert, Michael Synowitz, Peter Wend, Michael Fähling, Erich E. Wanker, Marie-Pierre Junier, Rainer Glass, Helmut Kettenmann and Jochen C. Meier. "Intracellular glycine receptor function facilitates glioma formation in vivo."
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Feng Hu*, Min-Chi Ku*, Darko Markovic*, **Omar Dildar a Dzaye**, Seija Lehnardt, Michael Synowitz, Susanne A. Wolf and Helmut Kettenmann. "Glioma associated microglial MMP9 expression is up regulated by TLR2 receptor activation and sensitive to minocycline."
International Journal of Cancer (**2014**): 24752463 (IF=5,085)

Omar Dildar a Dzaye, Suzy Cleator and Petros Nihoyannopoulos. "Acute coronary artery thrombosis and vasospasm following capecitabine in conjunction with oxaliplation treatment for cancer."
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