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Analysis of YKL39 and stabilin-1 as indicators for alternative activation of macrophages in tumor microenvironment

Autor: Bin Song
Institut / Klinik: Institut für Transfusionsmedizin und Immunologie
Doktormutter: Prof. Dr. J. Kzhyshkowska

Macrophages are key cells of innate immune system. Pathological activation of macrophages results in chronic inflammation, which leads to serious human pathologies, including solid tumors. The analysis of macrophage biomarkers allows the classification and identification of macrophages subpopulations in inflammation and in tumors. TGF-β is an important driver of pathological macrophage programming in cancer. Previous studies have demonstrated that TGF-β can stimulate expression of YKL-39 gene expression in macrophages. However the antibodies to examine YKL-39 expression in cells and tissues were not available. The aims of the current project included: 1) establishment of a test system for the analysis of specificity of newly generated anti-YKL-39 antibodies; 2) analysis of specificity of the newly generated anti-YKL-39 antibodies; 3) analysis of the TGF-β effect on the expression of YKL-39 in different subpopulations of human macrophages; 4) analysis of intracellular localization of YKL-39 in M2 macrophages and its co-localization with stabilin-1; 5) examination of expression of YKL-39 in TAMs subpopulations in human glioblastoma samples. The test system was designed by transient transfection of pcDNA3-YKL-39-FLAG into mouse breast adenocarcinoma cell lineTS/A. Immunization of mice, rat and guinea pigs wi th YKL-39 peptides has been performed in cooperation with Dr. H-R. Rackwitz (Peptide S peciality Lab, Heidelberg) and Dr. E. Kremmer (Helmholzzentrum, München). 43 murine hybridomas, 73 rat hybridomas and 4 sera from immunized guinea pigs were tested in this work using TS/A-YKL-39-FLAG and TS/A-vector control cells for the specific recognition of recombinant YKL-39. Monocytes were isolated out of buffy coats by gradient centrifugation and CD14+ positive selection. M2 subpopulations were generated by stimulation with IL-4 alone, IL-4+dexamethasone and IL-4+dexamethasone+TGF-β directly after monocyte isolation. The expression of YKL-39 protein was analyzed using quantitative RT-PCR, and by immunofluorescent staining/confocal microscopy using the newly generated antibodies. Using a-stabilin-1 RS1 antibody, co-localization of YKL-39 and stabilin-1 was studied in glioblastoma in combination with CD68, CD163, CD206, and LYVE-1 using triple immunofluorescent staining and confocal microscopy. Most of the anti-YKL-39 antibodies generated in mice specifically recognized YKL-39. Clones H6G4, H3E4, H5A2,14E4, 14C12, 14E10, 11H3, 15D12 and 24A6, which were strongly positive, were selected for the sub-cloning. The strongest clone 14E10 was selected for further experiments. A few of the anti-YKL-39 antibodies generated in rats specifically recognized YKL-39. Clones 13D7, 18H10, 14D7, H9A7 and H10G8 were strongly positive and were selected for the sub-cloning. The strongest rat mAb 18H10 was selected for further experiments. As for the YKL-39 antibodies generated in guinea pigs, the strongest serum (I4) was tested. The expression of YKL-39 mRNA was strongly upregulated in macrophages stimulated by the combination of IL-4+dexamethasone+TGF-β in three out of four donors. Only IL-4+dexamethason+TGF-β–stimulated macrophages expressed the endogenous YKL-39 protein, as detected by immunofluorescent staining and confocal microscopy using mYKL-39 and rYKL-39 monoclonal antibodies. In glioblastoma sections, YKL-39 was partially co- expressed with stabilin-1+ and LYVE-1+ in intratumoral macrophages. Stabilin-1 was partially expressed by CD68+ cells and expressed in majority of CD163+ or CD206+ cells, suggesting that stabilin-1 can be used as an M2 macrophage marker in glioblastoma. YKL-39 can be used as a potential biomarker of M2 macrophages in glioblastoma. Nevertheless, since YKL-39 is only partially co-expressed by stabilin-1+ CD68+ macrophages, it should be used in combination with other M2 markers. YKL-39 can serve as a biomarker for the reaction of TAMs in TGF-β in tumor microenvironment. Newly generated murine mAb 14E10 and rat mAb 18H10 were tested in immunohistology and demonstrated high specificity and intensity of thereactions and can be further used for the analysis of YKL-39 expression in patients' samplesand in in vitro experimentation.