

Is YKL-40 (CHI3-L1) a new possible biomarker prognosticator in high grade glioma?

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Abstract: A biomarker is "a naturally occurring molecule, gene, or characteristic by which a particular pathological or physiological process, disease, etc. can be identified" and it could be used a measurable indicator for the presence or severity of disease state. YKL-40 is a secreted glycoprotein associated with extracellular matrix, a member of the mammalian chitinase-like proteins that is expressed in a several types of solid tumors. Although the implication of this biomarker in tissue remodeling processes or the role in cancer cell proliferation, invasiveness, angiogenesis, and remodeling of the extracellular matrix is going to be well recognized, the regulation and role in glioblastoma multiforme (GBM) progression remains unknown. Using the serum level of YKL-40 as a single screening test in cancer cannot be used, but in association with other tumoral biomarkers and imaging techniques can be a useful tool as a "prognosticator." Moreover, elucidation of the YKL-40 functions could be an attractive target for antitumor therapy.

Key words: biomarkers, high grade glioma, YKL-40.

Introduction

In the last 10 years great efforts were made to find new biological markers in human pathology, and special attention was done to discover new tumoral markers that could help in defining new treatment possibilities, and to offer a better way to perform a prognosis with a high accuracy. High grade glioma represent an important group of human brain tumors, and in this respect finding new characteristics in their biology could open new ways in what represent challenges in therapy for this highly malignant with rapid evolution, and a dismal prognosis. The majority of the primary tumors of the central nervous system are represented by gliomas, and they are classified according to WHO into four different classes with the grade IV or glioblastoma multiforme (GBM) exhibiting the highest level of malignancy in terms of invasion, proliferation, angiogenesis,

and necrosis. GBM is one of the most common primary brain tumor, and the most fatal in terms of survival, meaning that the survival time is 10 to 14 months with the current standard of care, including surgical removal, radiotherapy, and chemotherapeutic agent temozolomide. The GBM cells aggressively infiltrates the surrounding brain tissue, and this is the cause of his very high recurrence rate.[1]

The complex nature of these tumors lead to classified GBM in subtypes, just in an attempt to guide research to develop new targeted therapies, listed in the best prognostic order as Neural, Pro-neural, Classical, and Mesenchymal. The Neural and Pro-neural resembles normal brain cells, in contrast with Classical and Mesenchymal subtypes that are much less differentiated and have a much worse prognosis.[2]

One of the most overexpressed proteins by GBM cells is YKL-40. Is a glycoprotein that

play a major role in the maturation of some cells of the immune system and its implications in inflammatory diseases and in some forms of solid tumors is growing. But, the exact mechanisms of YKL-40 in GBM remain unknown, and to validate it as a biomarker needs more work to be done.

CHI3-L1/YKL-40

In the last years a secreted glycoprotein associated with extracellular matrix, YKL-40, was identified to be the most highly up-regulated protein, and even more the microarray analysis offer the information that YKL-40 could be one of the most overexpressed genes compare to normal brain tissue, but the role in progression of GBM remains elusive.[1]

This glycoprotein in the light of the last years could represent a possible biomarker used in situation of patients with high grade gliomas (HGG), but according to the tumor marker utility grading system, a number of requirements must fulfilled before considering to have reached level of evidence I (LOE I) to be clinical implementation feasible. In the most situations, including YKL-40, the studies of biomarkers are at the level LOE III, defined as retrospective studies. The LOE II, an intermediate level, is defined as studies with prospectively collected specimens.[3]

The 18 glycosyl hydrolase family of chitinases is widely expressed from prokaryotes to eukaryotes, including mammals, despite the absence of endogenous chitin. Acidic mammalian chitinase (AMCase) play a multiple roles including inhibiting chitin-induced innate inflammation; augments chitin-free, allergen-induces Th2 inflammation; and mediates effector functions of IL-13. The YKL-40/chitinase-like proteins BRP-39 plays important roles in inhibiting oxidant-induced lung injury, augments adaptive Th2 immunity, regulates apoptosis,

stimulates alternative macrophage activation, and contributes to fibrosis and wound healing.[4]

YKL-40 was first discovered in human osteosarcoma cell line, MG-63, and is a 40 kDa protein, and its first three aminoacids - tyrosine, lysine, and leucine - giving his name. Even YKL-40 can bind to chitin directly, but the glycosyl hydrolase activity to break down chitin is absent.

The YKL-40 is expressed by activated neutrophils, macrophages, vascular smooth cells, and chondrocytes and play an important role in extracellular matrix remodeling, macrophage induced inflammation, and T-cell function. That's why the YKL-40, implicated in maturation of some cells of the immune system, especially macrophages, may act as a prognostic marker for inflammatory diseases and cancer, but the role of YKL-40 in relation to tumors development and progression still remains unknown, and more work must be done to validate it as a tumoral marker.

The location of gene encoding the YKL-40 is on chromosome 1q32.1, and chitinases (including YKL-40) are endo- β -1,4-Nacetylglucosamides are part of the innate immune response being involved in the host defense against organisms that contain chitin, which is found in the cell walls of many bacteria and fungi.[1]

YKL-40 SERUM LEVEL IN PATIENTS WITH TUMORS

The serum level of the YKL-40 is found elevated in patients with different types of solid tumors, including adenocarcinomas, small cell lung carcinoma, glioblastoma, and melanoma, with the highest level in patients with advanced cancer and the poorest prognosis, and in many cases could provides independent information of survival, but at this time he cannot be used as a single screening test for cancer.[3]

In vitro, YKL-40 is secreted by a number of cell lines, including the glioblastoma cell line U87 and the glioma cell lines U1242MG, U343MG and U1231MG. Microarray gene analyses in glioblastomas and astrocytomas showed that YKL-40 is overexpressed compared to normal tissue, and its expression increases with glioma grade, being higher in glioblastoma than in astrocytic or oligodendroglial tumors. This high expression of YKL-40 was associated with a poor response to radiotherapy and a short time to disease progression and death. For adult patients operated for HGG, plasma level of YKL-40 during follow-up was lower in cases with no radiographic evidence of recurrences compared to patients with signs of disease and was associated with short survival, but in pediatric HGG overexpression of YKL-49 is less frequent and is not correlated with survival.[5]

The results show that YKL-40 plays a pivotal role in proliferation of glioma cells through activation of the MAPK and AKT pathways [6], and suppression by shRNA reduced glioma cell invasion, anchorage-independent growth and increase cell death triggered by chemotherapy (e.g. cisplatin, etoposide and doxorubicin).[7]

YKL-40 AND ANGIOGENESIS

Tumor cell invasion and metastasis can be influenced by inhibition of angiogenesis, and blocking vascular endothelial growth factor receptor-2 (VEGFR-2) with monoclonal antibody DC101 inhibit GBM cell growth, but can increase tumor cell invasion using the preexistent vasculature. This increased invasion of the GBM cell can be inhibited by simultaneous blockade of epidermal growth factor receptor (EGFR).[8]

Clinical trials in recurrent GBM testing an anti-VEGF antibody show minimal benefit in terms of survival. Moreover, a knockdown of the VEGF gene in the U87 GBM cell line will

up-regulate YKL-40 levels, and remains to be determined the regulatory relationship between YKL-40 and VEGF, but both act as potent angiogenic factors in a synergistic effects on angiogenesis.[1]

The YKL-40 is not regulated by VEGF, and a long-term blockade of VEGF will have an angiogenic compensative activity of GBM cells by inducing YKL-40. The phenomenon of promoting vascular development observed in tumor models treated chronically with a single anti-angiogenic drug is known as angiogenic rebound.[9][10]

Studies demonstrated that YKL-40 could enhance angiogenesis, radioresistance, and progression of GBM cells, and in the end YKL-40 can drove GBM cells into mesenchymal phenotype, where the tumor cells act as mural-like cells.[2]

The receptors for YKL-40 still remains unknown, but YKL-40 induces interactions between integrin $\alpha_3\beta_1$ and syndecan-1 in endothelial cells, though syndecan-1 don't mediate migration and invasion of GBM cells induced by YKL-40. Other studies indicated that extracellular matrix is important for migration and invasion of the glioma cells, and YKL-40 can bind the heparin binding domain of syndecan-1, a transmembrane heparin sulfate proteoglycan in endothelial cells. However, syndecan-4 is at high levels in all glioma cells, and his down-regulation will reduced YKL-40-induced U373 cells migration.[1]

YKL-40 IN CLINICAL PRACTICE

Recently, YKL-40 was introduced into clinical practice, but its application remains restricted.[11] The serum level of YKL-40 and MMP-9 can be monitored to help confirm the absence of active disease in GBM and YKL-40 in anaplastic glioma patients.[12]

In a study made for YKL-40 expression in 36 patients with glial tumors and 33 age-

matched healthy persons, expression of YKL-40 was measured by gene analysis, immunohistochemistry and ELISA. In patients with HGG the YKL-40 serum levels was significantly increased compare to healthy subjects, and serum concentrations increased with tumor grade and correlated positively with transcript rate.[13]

A prospective longitudinal study made a correlation between the serum levels of YKL-40 and MRI findings in 197 patients with GBM. In patients with no radiographic evidence of disease the serum levels of YKL-40 was significantly lower compared to patients with radiographic evidence of tumor. Furthermore, a smaller study was made in 60 patients with GBM who underwent gross total resection or subtotal resection, and showed that in patients who had more extensive tumor resection the levels of YKL-40 dropped postoperatively. In a study of 105 patients, the most important prognostic factors in patients with primary GBM were the extent of resection, MGMT promoter methylation status and YKL-40 expression by immunohistochemistry.[14] Also, longitudinal increases in serum level of YKL-40 is associated with increased risk of death in patients with GBM or anaplastic gliomas.[15]

Glial fibrillary acidic protein is the current standard immunohistochemical marker used to differentiate between different types of gliomas, but in one study YKL-40 staining offer a better distinction of GBM versus anaplastic oligodendroglioma, and a combination of those two staining's offer even a greater diagnostic accuracy.[16] Despite identical histological features, the biology of HGG in children differs from that in adults.[17]

DOES THERAPY INFLUENCE YKL-40 SERUM LEVEL?

In a study of 60 patients who underwent a standard treatment (surgery, radiotherapy and chemotherapy) and standard radiological monitoring (MRI at pre-defined stages) the YKL-40 serum level was evaluated. Patients were divided in two groups based on the extent of resection (total or subtotal) in accordance with MRI results after 48 hours following surgery. At multivariate analysis a significant hazards ratio of 1.97 was found, and a significant association with shorter outcome in patients whose postoperative YKL-40 concentration increases higher than 100%; a negative prognostic index is still considered for a 50% increase. As a conclusion, biomarker YKL-40 could provide earlier and additional information and is a further aid in establishing the prognosis of GBM patients who undergone surgery.[18]

In another study, poorer radiation response, shorter time to progression and shorter overall survival was associated with higher YKL-40 expression in the subtotal resection group and it was validated in the gross-total resection group by association with higher YKL-40 expression. The YKL-40 was an independent predictor of survival, after adjusting for patient age, performance status, and extent of resection.[19]

A combination of preoperative profile IGFBP-2, GFAP, and YKL-40 plasma levels could serve as an additional tools for patients with inoperable brain lesions suggestive of GBM.[20]

The most important factor who influence the OS of patients with GBM is the extent of removal, and is an independent prognostic factor that predicts OS better than MGMT status.[21]

A mouse monoclonal anti-YKL-40 antibody (mAY) bind specifically with recombinant YKL-40 and with YKL-40 secreted from osteoblastoma cells MG-63 and brain tumor cells and thus inhibit tube formation of microvascular endothelial cells;

also abolish activation of the membrane receptor VEGF receptor 2 and intracellular signaling mitogen-activated protein kinase extracellular signal-regulated kinase (Erk) 1 and Erk 2; enhance cell death response of U87 line to γ -irradiation.[22] Thus the conjunction therapy with mAY and radiotherapy synergistically inhibit vascularization and progression in tumor.[23]

YKL-40 can be used as predictor of survival in patients with HGG, and longitudinal studies with a larger patient population are needed to confirm these findings.[12]

Conclusion

CAN WE USE YKL-40 AS A BIOMARKER IN GBM?

YKL-40, this 40kDa glycoprotein belonging to the chitinase family but without chitin hydrolase activity is a new possible biomarker in GBM. It acts as an antiapoptotic protein, but the exact mechanism in GBM progression, invasiveness and angiogenesis remains elusive.

According to Werner et al. there are six different clinical criteria to assess the value of tumor markers in clinical practice, such as biochemical characteristics, organ specificity, or clinical usefulness.[24] YKL-40 is neither organ- nor tumor-specific, and in the “tumor marker utility grading system” introduced by Hayes and colleagues is on the “utility scale +” and “utility scale +/-”, indicate that serum level of YKL-40 may have a role in screening and monitoring of patients with cancer.[25] The importance of YKL-40 in screening and monitoring of the patients with GBM needs to be proved by appropriate prospective study in which is assessed the benefit of using serum levels of YKL-40 in clinical decision-making process.

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