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Immunohistochemistry is a powerful method to assess mTOR activity in clinical/histological samples, however, care should be taken in choosing the targets for determining mTOR activity due to the complexity of its regulation.

Expression of both of these biomarkers was associated mostly with male gender. Further, older patients showed a trend towards having more mTOR positive tumors. Based on our results, we hypothesize that targeted therapy with mTOR inhibitors may have a role as an additional novel component in a subset of multiple myeloma patients. Overexpression of protein kinases, instrumental in the regulation of cell growth and proliferation, contributes to malignant phenotypes in a number of human cancers [2 , 3]. Integration of environmental signals, nutrients, and stresses stimulate mTOR to interact with partner proteins within each complex. The active kinase generates PIP3 at the lipid membrane. As the central effector of the PI3K pathway, Akt transmits signal to a host of downstream substrates, thus influencing a variety of key cellular functions. Pharmacological inhibition of the pathway is achieved through a variety of compounds in clinical use at various points along the pathway that are indicated by the red. Dysregulation of mTOR signaling has been identified in patients diagnosed with a number of human cancers, including MM, and has become a target for treatment in an effort to increase overall survival [7 , 8]. To the best of our knowledge, immunohistochemical expression of the mTOR pathway in multiple myeloma has not yet been studied and most of the work has been performed on cell lines. In this study, we evaluated immunohistochemical expression of mTOR and p-mTOR protein in paraffin embedded tumor samples bone marrow biopsies and clot sections from 31 multiple myeloma patients. The expression of these biomarkers was further correlated with cytogenetic results and clinical parameters. In a new window Figure 2 A Bone marrow biopsy with focal paratrabecular plasma cell infiltrate, normal bone marrow hematopietic particles and marrow fat. For mTOR and p-mTOR, breast tissue served as a positive control with additional slides of normal tonsil tissue and normal bone marrow which were used as baseline negative controls. As the m-TOR pathway is not activated in normal hematopoietic stem cells and normal bone marrow tissue shows no expression of m-TOR protein, the foci of normal marrow present in each the bone marrow biopsy or clot sections used for immunostaining served as additional internal negative controls [11 , 12]. Immunohistochemical stain analysis Two observers independently reviewed and interpreted the immunohistochemical stains on all cases. For comparison with clinical variables, the expression level of the biomarker was semi-quantified by multiplying the scores of staining intensity and percentage of positive tumor [13]. Comparisons of mTOR and p-mTOR expression to clinical variables and cytogenetic results were done with expression of these biomarkers as dichotomized variables classified as positive or negative. Multivariate analysis was not performed due to small sample size. Results Demographics Our study group consisted of 13 males The mean age of the patients was Table 1 lists the demographic data and baseline characteristics of the study group.

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mTOR is a key regulator of cell growth and size, and its activity is often dysregulated in a wide variety of diseases. The mTOR signaling pathway is also a therapeutic target for many diseases.

The primary treatment modality for OSCC is surgery. Recent advances in treating OSCC have led to the improvement of local tumor control, but fewes than half of patients with advanced OSCC survive for 5 years. Therefore, new treatment strategies are required 1. Although angiogenesis, the formation of new blood vessels, is a normal physiological event, it is closely related to both tumor growth and metastasis. These newly-formed vessels provide the principal route by which tumor cells exit the primary tumor site and enter the circulation. For many types of tumors, vascular density is a prognostic indicator of metastatic potential, with highly vascular primary tumors having a higher incidence of metastasis than poorly vascularized tumors. We now know there are various molecular determinants of these different mechanisms of vascular growth 2 - 5. Among them, VEGF-A is known to be a key angiogenic factor, and is the most frequently used by a tumor to switch on its angiogenic phenotype 7. In fact, VEGF-A overexpression has been reported in most types of cancer, including oral cancer, and it is thought to be a prognostic factor for survival 8 - VEGF-C has been detected in several different types of cancer, and its level in some studies seems to correlate with nodal metastasis and patient survival 10 , 15 , Recently, various drugs targeting VEGF-mediated signaling have been introduced into the treatment of terminal cancer to control tumor angiogenesis at the clinical level 17 - Several reports have indicated that tumor cells under hypoxic conditions have high neovascularity and aggressive behavior. Although cell growth is generally decreased under hypoxic conditions, some tumor cells overcome inhibition of proliferation and adapt to growth under hypoxia. In a new window Figure 1. The aim of this study was to clarify the relationship between VEGF expression and clinicopathological factors. All samples from OSCC patients were biopsy specimens. The specimens of normal oral mucosa from 10 healthy individuals were used as controls. Tumor histological differentiation was defined according to the World Health Organization classification 27 and the invasive grade was assessed by the Yamamoto-Kohama YK mode of invasion In a new window Figure 2. Endogenous peroxidase was blocked by incubation with 0. Results were evaluated by calculating the total immunostaining score as the product of the proportional score and the intensity score. The intensity score represents the estimated staining intensity 0, no staining; 1, weak; 2, moderate; 3, strong. The total score ranges from Immunohistochemical overexpression was defined as a total score greater than 4, since the patient samples showed a bimodal distribution of immunohistochemical expression with the discriminating nadir at a total score value of 3 to 4. Significance was assessed by the Mann-Whitney U-test. Survival analysis was calculated by the Kaplan-Meier method and compared using the log-rank test. The proteins were found to be strongly expressed in the invasion front of the tumor. Representative immunohistochemical staining is shown in Figure 1. Immunohistochemical expression of CD34 represents a good method to quantify angiogenesis in carcinoma.

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Chapter 3 : mTOR: Methods and Protocols: Thomas Weichhart | NHBS Book Shop

Immunohistochemical analysis of mTOR activity in tissues more by Jinhee Kim mTOR is a key regulator of cell growth and size, and its activity is often dysregulated in a wide variety of diseases.

Received Sep 17; Accepted Dec This article has been cited by other articles in PMC. The dysregulation of the mTOR pathway has been found to be a contributing factor of a variety of different cancer. To investigate the role of mTOR signal pathway in the stepwise development of gastric carcinomas, we analyzed the correlations between the mTOR and P70S6K expression and clinic pathological factors and studied its prognostic role in gastric carcinomas. Conclusion Aberrant expression of p-P70S6K possibly contributes to pathogenesis, growth, invasion and metastasis of gastric carcinomas. It was considered as a promising marker to indicate the aggressive behaviors and prognosis of gastric carcinomas. It continues to be a major health problem because of the slow decrease in incidence in Asia and high mortality of diagnosed gastric carcinoma in West [1]. Therefore, it is of much significance for the prevention, treatment and prognosis evaluation of gastric cancer to clarify its molecular mechanisms and find out a good biomarker to indicate its carcinogenesis and subsequent progression. This kinase is controlled by multiple phosphorylation events located within the catalytic, linker and pseudosubstrate domains and subsequently phosphorylates specifically ribosomal protein S6. Activation occurs via phosphorylation at ser, Thr and Ser within the pseudosubstrate region. Phosphorylation of Thr in the catalytic domain and Thr in the linker domain are most critical for kinase function. Stimulation of mammalian cells by a variety of mitogenic stimuli results in a rapid, biphasic activation of p70S6K. In the present study, we observed that mTOR and P70S6K expression were examined in gastric carcinoma, adjacent non-tumorous mucosaand adenoma, and compared with the clinicopathological parameters of tumors to explore the clinicopathological significance and molecular role of the mTOR signal pathway in the stepwise development of gastric carcinomas. Among them, cases have carcinomas accompanied with lymph node metastasis. None of the patients underwent chemotherapy or radiotherapy before surgery. They all provided consent for use of tumour tissue for clinical research and our University Ethical Committee approved the research protocol. We followed up all patients by consulting their case documents or through telephone. These sections were stained by haematoxylin-and-eosin HE to confirm their histological diagnosis and other microscopic characteristics. The staging for each gastric carcinoma was evaluated according to the Union Internationale Contre le Cancer UICC system for the extent of tumour spread [12]. Furthermore, tumour size, depth of invasion, lymphatic and venous invasion were determined. HE staining was performed on TMA for confirmation of tumor tissue. Y, , Epitomics, USA; 1: E, , Epitomics, USA; 1: Omission of the primary antibody was used as a negative control. One hundred cells were randomly selected and counted from 5 representative fields of each section blindly by three independent observers. The positive percentage of counted cells was graded semi-quantitatively according to a four-tier scoring system:

Chapter 4 : Tissue expression of MTOR - Summary - The Human Protein Atlas

In mTOR: Methods and Protocols expert researchers in the field detail many of the methods which are now commonly used to study mTOR. These include methods and techniques used for the study of the mTOR pathway and potential therapeutic applications of mTOR inhibitors such as, immunosuppressive and anticancer agents.