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Analysis of Proteoglycans Gene Expression in non Small Cell Lung Carcinoma

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INTRODUCTION

Lung cancer is of the leading causes of cancer deaths among the industrialized countries. Non small cell lung cancer (NSCLC) accounts for the 80% of the different lung cancers. 40% of the individuals operated for NSCLC and verified post operationally tumor free relapse within the next 24 months. Moreover individuals histologically subtyped in the same stage, exhibit different course of disease. This suggests despite of the same histological type, NSCLCs have different biological background. Despite of the intense research the critical pathways concerning growth and invasion of tumors remain unclear. Proteoglycans (PGs) are macromolecules consisting of glycosaminoglycan chains covalently linked to a protein core. They comprise a superfamily numbering more than 30 members with diverge biological functions. Such functions include modulating of growth factor activities and influence cell growth and invasion of tumors. Aggrecan and Versican belong to a small family of PGs present in the extracellular matrix. Abnormal versican expression has been demonstrated in colon carcinoma and it has been shown that it is overexpressed in the stoma of different tumors. The role of Aggrecan in cell growth is still unclear. Decorin belongs to the small leucine rich protein (SLRP) proteoglycan family. It has been shown that it can act as an extracellular reservoir for the TGF-β thus modulating its activity. Decorin is recognized by the EGF receptor activating the MAP signal transduction pathway leading to intracellular increased levels of p21. p21 as a potent inhibitor of cyclin depended kinases causes growth arrest.

AIM

The aim of this work is to investigate the gene expression for these proteoglycans in NSCLC and assess their role concerning the biology of the cancer.

MATERIALS AND METHODS

Tumor and surrounding healthy tissue were obtained form individuals that underwent surgery for NSCLC. Tissues were histologically classified as NSCLC squamous or adenocarcinoma, frozen in liquid nitrogen and stored in -70°C until use. Total RNA was isolated with the RNeasy Mini RNA extraction kit from Qiagen. Total RNA was reverse transcribed with MMLV-RT and amplified for α) Versican as well as for the EGF-like and CRP-like splisomeres β) for the EGF-like and CRP-like splisomeres of Aggrecan and γ) for Decorin. Amplification was achieved by an initial step of denaturation at 94°C for 5 min. and 30 cycles of 30 sec denaturation at 94°C, 30 sec annealing at the Tms of the primers and 1min extension at 72°C. Amplification products were analyzed on a 2% agarose gel. The relative levels of expression between normal and tumor tissue of the same individual were established by using Quantum RNA 18S from Ambion.

RESULTS

Aggrecan mRNA is expressed both in normal and tumor tissue. Both the EGF-like and CRP-like splisomeres are present. The mRNA levels for aggrecan both in normal and tumor tissue of the same individual seem to be comparable. Versican mRNA is heterogeneous consisting of different EGF-like and CRP-like splisomeres. Versican mRNA is present both in the normal and tumor tissues. It is spliced only for one EGF-like domain and for the CRP-like domain. The degree of splicing among matched tissues appears to be the same. In squamous NSCLC the expression of versican mRNA is increased compared to the healthy tissue whereas in adenocarcinoma the expression of versican is abnormal. Decorin mRNA expression is decreased in squamous as well as adenocarcinoma when compared with the healthy tissues.

CONCLUSIONS

Genes coding for aggrecan, versican and decorin express in NSCLC. The level of versican mRNA expression is elevated in the squamous NSCLC whereas we observe a drastic reduction in the level of mRNA expression for decorin both in squamous and adenocarcinoma NSCLC. The fact that decorin through the MAP signaling pathway ultimately controls cell growth correlates with its decreased levels of expression in NSCLC. Our observations implicate these molecules for the first time in the biology of non small cell lung cancer and can be of value elucidating the molecular mechanisms governing cell growth and invasion of tumors.