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INSULIN-LIKE GROWTH FACTORS/RECEPTORS IN LUNG CANCER

JASMINKA PAVELIĆ

Division of Molecular Medicine, Rudjer Bošković Institute, Zagreb, Croatia

Summary

Perturbation in a level of any peptide from insulin-like growth factor (IGF) family (ligands, receptors, binding proteins) seems to be implicated in lung cancer formation; IGF ligands and IGF-R1 through their mitogenic and anti-apoptotic action, and the IGF-R2/M-6-P possibly as a tumor suppressor. In this respect we have found that human lung cancers overproduce IGF-1, IGF-2 and IGF-R1, which in turn stimulates their proliferation by autocrine mechanism, decrease apoptosis rate and increase telomerase activity. At the same time majority of tumors underproduce IGF-R2 possibly due to the mutations in both alleles of this gene. However, cancer cell proliferation can be abrogated or alleviated by blocking the mRNA activity of IGF-1, IGF-2 and/or IGF-R1 genes indicating that the use of monoclonal antibodies or an anti-sense approach may represent an effective and practical cancer gene therapy strategy.

KEY WORDS: *insulin-like growth factors, receptors, lung cancer*

INSULINU SLIČNI ČIMBENICI RASTA/RECEPTORI KOD RAKA PLUĆA

Sažetak

Velike promjene vrijednosti bilo kojeg peptida iz porodice insulinu sličnih čimbenika rasta /IGF/ (ligandi, receptori, vezujući proteini) čini se imaju utjecaja na nastanak raka pluća; IGF ligandi i IGF-R1 svojim mitogeničnim i anti-apopstotskim djelovanjem, a IGF-R2/M-6-P vjerojatno kao tumorski supresor. U tom pogledu otkrili smo da rak pluća u ljudi proizvodi previše IGF-1, IGF-2 i IGF-R1, čime se pak potiče njihovo širenje pomoću autokirnog mehanizma, smanjuje brzina apoptotskog procesa i povećava aktivnost telomeraze. Istodobno većina tumora proizvodi premalo IGF-R2, vjerojatno zbog mutacija prisutnih u oba alela toga gena. Međutim, širenje stanica raka moguće je prekinuti ili usporiti blokadom aktivnosti mRNA gena IGF-1, IGF-2 i/ili IGF-R1, što upućuje na to da bi primjena monoklonskih protutijela ili tzv. antisense pristupa mogla biti učinkovita strategija upotrebljiva u genskoj terapiji raka.

KLJUČNE RIJEČI: *insulinu slični čimbenici rasta, receptori, rak pluća*

INTRODUCTION

Insulin, insulin-like growth factors (IGF's), as well as later discovered insulin-like growth factor receptors (IGF R of type 1 and type 2) and insulin-like growth factor binding proteins (IGF-BP 1-6) (IFG family of peptides), play an important role in the normal control of many metabolic and growth related processes. They have been shown to have mitogenic and distinct apoptotic effects regulating thereby the growth of mammalian organism; they can act in endocrine (like a hormone), and autocrine/paracrine manner. Consequently, their disruption could trigger mechanisms that lead to human carcinoma development.

IGF-1 AND IGF-2

IGF-1 is a trophic factor that circulates at high levels in the blood stream. Although the main source of IGF-1 in the serum is the liver, many

other tissues synthesize it and are sensitive to its action, especially during postnatal development (1). Regulation of hepatic IGF-1 production is mostly mediated by growth hormone and insulin. In turn, IGF-1 feeds back to suppress growth hormone and insulin release. In addition to growth hormone, developmental factors as well as nutrition status all modify IGF-1 production (1).

The synthesis of IGF-2 is relatively growth hormone independent. Its expression is much higher during fetal development than in postnatal life. It acts as a regulatory peptide; it is mitogenic for a number of cell types. The IGF-2 gene can be expressed to produce proteins of various molecular weights. The most active form, with regard to binding to IGF receptors, is 7.5 kDa (2). Larger forms lack posttranscriptional cleavage and have been implicated in hypoglycemia, which can accompany a variety of tumors.

INSULIN-LIKE GROWTH FACTOR RECEPTORS

The biological effects of IGF-1 and IGF-2 on a target cell are mediated by two types of cell surface receptors: IGF receptor of type 1 (IGF-R1) and IGF receptor of type 2 (IGF-R2), as well as through binding to receptors for insulin. IGF-1 binds to the type 1 receptor, and with a lower affinity to insulin receptor. IGF-2 binds with high affinity to the type 2 receptor and with low affinity to the type 1 receptor. It has no affinity for the insulin receptor. The type 2 receptor is identical to the cation-independent mannose-6-phosphate receptor. In general, most of the action of IGFs is mediated via IGF-R1 (1). It is a member of tyrosine-kinase class of growth factor receptors. Binding of a ligand (IGF-1 and IGF-2) to the extracellular part of IGF-R1 initiates a cytoplasmic signal cascade that stimulates downstream signaling through intracellular networks regulating cell proliferation, differentiation, migration and protection from apoptosis.

The type 2 IGF receptor, also known as cation-independent mannose-6-phosphate receptor (IGF-R2/M-6-P), is structurally and functionally different from the IGF-R1. The receptor is a monomeric membrane spanning glycoprotein which binds M-6-P, lysosomal enzymes, and IGF-2 (3). IGF-R2/M-6-P is involved in the clearance of IGF-2 from the circulation (at the cell surface the IGF-R2 is constitutively endocytosed, where its main role is the binding and internalization of IGF-2) and in the modulation of trafficking of lysosomal enzymes.

IGFS AND CANCER

During the past two decades, the joint efforts of several laboratories firmly established the important role of IGF family of peptides (ligands, receptors, binding proteins and proteases) as mitogens for variety of tumor types. As their action is strongly interrelated, any deregulation of interactions among them may lead to a pathological condition, mostly cancer formation and progression. In this respect, by tumor cells overproduced IGF-1 and IGF-2 very often act as autocrine stimulators of malignant cells division, through binding and stimulating the activity of IGF-R1. SCLC cell lines secrete and respond to exogenous IGFs, indicating an autocrine role for IGF-peptides in cancer cell proliferation. According to Reeve et al (4), approximately 50% of SCLC and 30% of NSCLC cell lines show IGF-2 gene expression. Lung tumor cell lines also secrete IGF-BPs (4-6). These proteins bind the IGFs and modulate the physiological and cellular actions of these peptides. Lung cancer also demonstrates loss of imprinting at the IGF-2 locus (7).

An alteration of the IGF-R2/M-6-P gene is also involved in lung cancer development. IGF-R2/M-6-P loss of heterozigosity (LOH) coupled with an intragenic loss-of-function mutation in the remaining allele, has been found in squamous cell carcinoma of the lung (8). An adenine-to guanine transition at exon 40 was also found in one lung adenocarcinoma cell line resistant to growth inhibition by TGFβ (9). Finally, LOH at the IGF-R2/M-6-P locus predisposes patients to radiation-induced lung injury (10). Thus, loss of function of this receptor could significantly alter normal cell growth.

Maybe the most important proof of the involvement of IGFs in tumor growth and progression comes from the clinical studies. For instance, several studies have shown the link between serum concentration of: 1) IGF-1 and IGF-BP 3 with increased risk of breast, prostate, colorectal and

lung cancer (11); 2) IGF-2 with increased risk of colorectal cancer (12). Overexpression of IGF-2, as measured at the level of mRNA and protein, is also found in a variety of cancers, including lung, gastric cancer and hemangiopericytomas (13-16).

Taking into account that some lung cancer cells produce IGF-R1 and IGF-2, which in turn stimulate cell proliferation by an autocrine mechanism, and that cancer cell proliferation can be abrogated or alleviated by blocking the mRNA activity of these genes, we have investigated the consequences of function/dysfunction of the IGF's family of genes on the behavior of different types of lung cancers.

In the first study (14), we have shown that the majority of human lung cancers tested (all together 69) overexpress simultaneously IGF-1, IGF-2, IGF-R1 and IGF-BP 4. Seventeen tumors were concomitantly positive for all four IGFs, whereas 34 were positive for IGF-2, IGF-R1 and IGF-BP 4 mRNA. An elevated amount of IGF-2 peptide was secreted into growth medium of cell cultures established from five different IGF-2/IGF-R1 mRNA positive lung cancer tissues. The cells also expressed elevated numbers of IGF-R1. Nine IGF-2 negative and 19 IGF-2 positive lung cancer of different stages were selected, and IGF-R2/M-6-P receptor was determined immunohistochemically. Most of the IGF-2 negative tumors were strongly positive for IGF-R2/M-6-P. IGF-2 positive tumors were mostly negative for IGF-R2/M-6-P. Antisense oligonucleotides to IGF-2 significantly inhibited, by 25-60%, the in vitro growth of all six lung cancer cell lines. However, the best results (growth inhibition of up to 80%) were achieved with concomitant antisense treatment (to IGF- R1 and IGF-2). These data suggest that lung cancer cells produce IGF-R1 and IGF-2, which in turn stimulated their proliferation by autocrine mechanism. Cancer cell proliferation can be abrogated or alleviated by blocking the mRNA activity of these genes indicating that an antisense approach may represent an effective and practical cancer gene therapy strategy.

In another study (16) we have tested a total of 38 human lung carcinomas (15 adenocarcinomas, 19 large-cell carcinomas, and four squamous cell carcinomas). Particularly, we investigated whether: 1) IGF-1/IGF-R1 mRNA/protein expression influence the intensity of cell proliferation and apoptosis; 2) genetic changes in IGF-R2/M-6-P gene influence tumor cell proliferation; 3) telomerase activity in tumor cells could be modulated by changing the activity of either IGF-1 or IGF-R1; and 4) disruption of IGF's function could influence lung cancer cell growth. A correlation between increased expression (at mRNA and protein levels) for both, IGF-1 and IGF-R1, and decreased apoptosis was found in large cell carcinomas and adenocarcinomas. In 40% of informative adenocarcinomas, expressing the highest values of IGF-2 and Ki-67 proteins, IGF-R2/M-6-P gene had LOH at one and a mutation in another allele. All four squamous cell carcinoma samples also expressed LOH/mutation in the IGF-R2/M-6-P gene. The αIR3 strongly diminished proliferation and increased apoptosis in cultures established from squamous cell carcinomas overexpressing IGF-2 and IGF-R1. Telomerase activity was followed in four squamous cell carcinomas. Cell treatment with IGF-1 increased telomerase activity. The opposite was observed when the cells were treated with αIR3 that inhibits the activity of IGF-1 receptors. Our findings suggest that disruption of the IGFs/IGF receptors axis is involved in lung cancer formation.

CONCLUSIONS

As the defects in IGFs (leading to a phenotype of anchorage-independent tumor growth) that are commonly seen in cancers are mostly associated with overexpression of IGF-1/IGF-2 and/or IGF-R1 as well as mutations in the IGF-R2 gene, targeting at the level of these four molecules would be a reasonable choice for the treatment of IGF-dependent cancers.

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Author's address: Professor Jasminka Pavelić, Division of Molecular Medicine, Rudjer Boškovic Institute, Bijenička 54, HR-10 002 Zagreb, Croatia, PO Box 180, E-mail: jpavelic@irb.hr