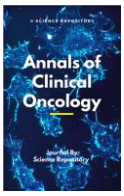

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Research Article

Serum carcinoembryonic antigen (CEA) levels are associated with the decrease/loss of p27 immunohistochemical expression in non-small cell lung cancer. Preliminary results

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ABSTRACT

Objective: to study the possible relation between immunohistochemical expression of p27 and serum carcinoembryonic antigen (CEA) levels in patients with non-small cell lung cancer (NSCLC)

Material and Methods: The study group included 35 patients with NSCLC (15 squamous and 20 adenocarcinomas). Immunohistochemical expression of p27 was studied through the technique of tissue-matrix using Tissue Arrayer Device, with monoclonal antibody M-T247 (Dako. Denmark). CEA serum levels were assayed using the "ECLIA" from Roche (Swiss).

Results: P27 expression was noted in 27 cases (77,1%), being slightly (+) in 14 cases and strong (++) in 13 cases. CEA serum levels were higher (p:0,026) in + positive carcinomas (range: 2,2-570,7; median 7,6 ng/ml) than in ++ positive carcinoma (range: 1,5-35,1; median 2,7), but there were not differences between + positive and negative carcinomas. Likewise, there were statistically significant differences (p:0,043) between ++ positive group and -/+ group (r:0,8-766,7; median 6,3 ng/ml).

Conclusions: Our results, preliminary do to the reduced number of patients included in the study, suggest that in patients with NSCLC, serum carcinoembryonic antigen levels can be associated with the decrease/loss of p27 immunohistochemical expression, important biological feature observed in various tumors, included lung carcinomas.

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Introduction

Cyclin dependent kinase inhibitor 1B (P27kip1; CDKN1B) regulates cellular proliferation and senescence. The gene encoded protein binds to and prevents the activation of cyclin E-CDK2 or cyclin D-CDK4 complexes, blocking G1/S transition and controlling the cycle cell in all organs [1, 2]. In this regard, some group has observed that SIRT1, a class III histone deacetylase, is an important regulator of p27kip1 expression, reducing its expression through the ubiquitin-proteasome pathway [3]. Likewise, SIRT inhibition induced senescence and anti-growth potential in lung cancer in vivo.

p27 has a role in tumor suppression, proliferation, cell migration and mitosis, and low p27 expression has been described in different malignant tumors, including lung carcinomas [4]. In lung cancer, reduced p27 expression is associated with tumor progression during the development of pulmonary carcinoma and was related with other prognostic and predictive parameters of poor prognosis as increased tumor size, lymph node metastasis high grade, early relapse, and reduced overall survival being an independent prognostic factor correlating with the overall survival [5-11]. Likewise, p27 plays an important role in the response to different treatments [12, 13].

Carcinoembryonic antigen (CEA, CEACAM5) is an oncofetal cell surface glycoprotein widely used in clinical practice as a tumor marker due to its high expression in some tumors and secretion to biological fluids, preferently serum. In non-small cell lung cancer (SCLC) CEA serum levels prior to surgery are associated with tumor size, lymph node, clinical stage and the histological subtype adenocarcinoma, as well as with brain metastasis in advanced tumors [14, 15]. Likewise, CEA with SCC and Cyfra 21.1 serum levels can be useful for differentiation of early-stage NSCLC from benign lung disease [16]. Also, CEA levels were a useful prognostic marker for either overall survival, recurrence after surgery or progression free survival, especially in adenocarcinoma subtype [17]. In patient's non-smokers with adenocarcinoma EGFR mutations are associate with a significantly higher incidence of abnormal CEA serum levels > 5 ng/ml [18]. In tissue samples, high CEA expression was observed more frequently in adenocarcinomas than in squamous cell carcinomas and was an independent prognostic factor for overall and disease-free survival by some groups [19].

In this work we have studied the possible association between CEA serum levels and p27 immunohistochemical expression in patients having non-small cell lung cancer.

Material and Methods

The study group included 35 patients (32 males), aged between 43 and 80 years (median 65) having non- small cell lung carcinomas (NSCLC; 20 adenocarcinomas and 15 squamous cell carcinomas). According to clinical stage, the patients were classified as follows: 3 IA, 9 IB, 3 IIA, 7 IIIA, 5IIIB and 8 IV; according to histological grade they were classified as follows: HG1: 3; HG2: 17 and HG3: 15. Blood samples were obtained two to five days before surgery, early in the morning in patients who fasted overnight. CEA serum levels were assayed using an electrochemiluminescence immunoassay ("ECLIA") from Roche (Swiss), with two monoclonal antibodies. The lowest limit of sensitivity was established in 0,2 ng/ml.

Lung carcinoma tissue samples were obtained at the time of surgery. Tissue slices had been fixed in 10% formalin and embedded in paraffin wax for histological and immunohistochemical studies. We used a Tissue Arrayer device (Beecher Instruments, Sun Prairie, WI) to construct two different TMA blocks, according to conventional protocols [20]. All cases were histologically reviewed, and the most representative areas were marked in the paraffin blocks. Two selected 1-mm-diameter cylinders from two different areas were included in each case from 35 carcinomas. All cases were from the files of the Department of Pathology in our hospital. Internal and external controls were included in each TMA. The immunohistochemical study was performed on 4-micron paraffin sections, using the Kit with universal secondary antibody that included a labeled-dextran polymer (DAKO EnVision Peroxidase/DAB; Glostrup. Denmark) to avoid the false positive reaction due to endogenous biotin activity. At least 500 cells were evaluated. Based on the percentages of immunopositive cells, three subdivisions were made as follows: diffusely positive (++), greater than 30% of cells were positive; heterogeneously positive (+), 10–30% of cells were positive; and negative (−), less than 10% of the cells were positive. Equivocal immunointensity was considered to be negative. We used the monoclonal antibody against p27 (Clone M-T247-dilution 1/20. Dako. Denmmark).

Data obtained were evaluated using the SPSS 15.0 software for Windows (SPSS, Chicago, IL. USA). With the parameters that did not follow a normal distribution, values were presented as range, and median. We used the Chi square test with Yates correction, if necessary, for qualitative variables comparison and the Mann Whitney test for continuous ones. A p-value ≤ 0.05 was considered as statistically significant.

Table 1: CEA serum levels (ng/ml) distribution according to p27 immunohistochemical expression in NSCLC patients

Subgroup	Range	pt25	median	P75
Negative	0,7-766,6	2,8	5,2	13,5
+ positive	2,2-570,7	2,8	7,6	9,2
++ positive	1,5-35,1	2,4	2,7	5,0
+/++ positive	1,5-570,7	2,5	5,2	9,1
-/+ positive	0,7-766,6	2,8	6,3	9,1

+ vs ++: p:0,026
++ vs -/+: p:0,043

Results

P27 immunohistochemical expression was noted in 27 cases (77,1%), being slightly (+) in 14 cases (40%) and strong (++) in 13 cases (37,1%). As you can see in Table 1, CEA serum levels were higher (p:0,026) in + positive carcinomas (range: 2,2-570,7; median 7,6 ng/ml) than in ++ positive carcinoma (range: 1,5-35,1; median 2,7), but there were not differences between + positive and negative (range: 0,7-766,7; median 6,2) carcinomas. Likewise, there were statistically significant

differences (p:0,043) between ++ positive group and -/+ group (r: 0,8-766,7; median 6,3 ng/ml).

CEA serum levels higher than 5 ng/ml were noted more frequently (p:0,049) in + positive subgroup (9/14) than in ++ positive tumors (3/13). Also, those were more frequently in -/+ subgroup (13/22) than in ++ subgroup (3/13), reaching statistical signification (p:0,057).

Discussion

P27kip1 is a negative regulator of cell proliferation and its expression is controlled by multiple transcriptional and posttranscriptional mechanisms, included some miR. Recently, Fernandez et al. have described that miR-340, a novel tumor-suppressor in NSCLC, correlated with the accumulation of p27 in lung adenocarcinoma [21]. Likewise, cells lacking the tumor suppressor p27 can be reprogrammed into induced pluripotential stem cells [22, 23]. From the clinical point of view, low p27 levels are associated with a poor outcome and a high tumor grade, being prognostic and predictive factor [24].

P27 immunohistochemical expression was noted in 27 cases (77,1%), being (+) in 14 cases (40%) and (++) in 13 cases (37,1%). Our results are similar to those described by other authors as Miao et al. whose described a total and nuclear positive rate of 72,5% and 46% respectively, lower that those observed in non-cancerous lung disease (94,3% and 94,3% respectively), Catzavelos et al. whose noted reduced levels in 86% of NSCLC, and Dobashi et al., whose described 89,2% of positive expression [2, 7, 25].

CEA serum levels were higher in + positive carcinomas than in ++ positive carcinoma, but there were not differences between + positive and negative carcinomas. Likewise, there were statistically significant differences between -/+ vs ++ patients when 5 ng/ml was used as threshold.

Our results, preliminary do to the reduced number of patients included in the study, led us to considered that in patients with non-small cell lung cancer, serum carcinoembryonic antigen levels are associated with the decrease/loss of p27 immunohistochemical expression in NSCLC, reflecting the biology of these malignant tumors, where the p27 degradation is involved.

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