Fas Expression is Associated with a Better Prognosis in Laryngeal Squamous Cell Carcinoma

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Abstract. Background: The role of apoptosis, and more specifically the Fas pathway, is still unclear in squamous cell laryngeal cancer. Materials and Methods: By means of immunohistochemistry, the expression of three genes involved in different pathways of apoptosis (p53, Bcl-2 and Fas) was studied in a series of 45 squamous cell laryngeal cancers from Spanish patients. Results: Of the studied tumors, 25 (55.6%) expressed mutant p53, 22 (48.9%) expressed Bcl-2 and 25 (55.6%) expressed Fas. p53, Bcl-2 and Fas expression were not correlated with each other. The only tested factors associated with significantly better survival of the patients were low tumor stage (p=0.0074), the absence of metastatic regional nodes (p=0.0009) and Fas expression (p=0.029). Fas expression was not correlated with the expression of any other tested parameter. Conclusion: The determination of Fas expression in squamous cell laryngeal carcinoma by means of immunohistochemistry using the GM30/CD95 antibody could be of potential use in the clinic.

Aptosis, or programmed cell death, plays a major role in tumor biology. Tumor cells differ from normal ones in that they are able to survive beyond their expected lifespan. Therefore impairment of apoptosis is the key mechanism in this context. Two major pathways regulate tumor cell apoptosis. The first is the so-called intrinsic pathway, mediated by mitochondria, in which the BCL family of genes, among others, is importantly involved; the second is the extrinsic pathway, initiated by the binding of a specific ligand, FasL, to a cell surface receptor, FAS (also known as APO-1 or CD95), belonging to the superfamily of tumor necrosis factor receptors. Both pathways converge activating several members of the caspase family, which are the ultimate triggers of apoptosis. The p53 gene is also involved in the mechanism of apoptosis, mainly by repressing genes involved in antiapoptotic functions (1, 2).

The role of apoptosis in squamous cell laryngeal carcinoma is still unclear. In the present investigation, we have studied by means of immunohistochemistry the expression of three genes representative of the above-cited pathways of apoptosis, namely p53, BCL-2 and FAS, in a series of squamous cell laryngeal cancers from Spanish patients, operated upon by the same surgical team at a single center, all of whom were subsequently followed-up until their demise or for a time span sufficiently large to allow for a statistically useful survival analysis.

Materials and Methods

Forty-five squamous cell laryngeal carcinomas from patients operated upon at the Hospital Nuestra Sra. Del Prado, Talavera de la Reina, Spain, were studied. The mean age of the patients was 62.9 years (range: 42-81). The surgery was performed by the same team throughout the study. The distribution by stages was as follows: T1: 2; T2: 15; T3: 21 and T4: 7. Fourteen tumors were histological grade 1, 26 grade 2 and 5 grade 3. The regional lymph nodes were invaded in 13 cases.

All patients were followed-up at the hospital. The mean follow-up time for surviving patients was 64.5 months (range: 12-153 months). Fourteen patients died during the follow-up period (range: 11-96 months after their initial surgery).

Immunohistochemistry. The immunohistochemical technique was the standard one used at our laboratory. The corresponding protocols have been extensively described in previous papers (3-5). For the present study, the bcl-2/100/D5 and the NCL-FAS-310 (clone GM30) monoclonal antibodies from Novocastra, Newcastle, UK, were used, both at a dilution of 1:50 after pretreatment of the slides with citrate buffer in a pressure boiler ("HIER" technique). The incubation was carried out for 1 h at room temperature in a humid chamber. As positive controls, normal breast tissue reactive for BCL-2 and small intestine for Fas were used, and as negative controls, duplicates were used from the tumors studied in each batch for whom all steps were carried out in parallel exactly in the same way, but omitting the first antibody. The slides were also routinely processed for hormone

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receptors (estrogen and progesterone), c-erb-B2, p53 and Ki-67 as described elsewhere (3-5). For the evaluation of the BCL-2 and FAS immunohistochemical reaction (Figure 1) a semiquantitative scale developed by us was used: this grades the tumors from 0 to 6, depending on the number of tumor cells stained and the intensity of the reaction in relationship to the positive control. The tumors were considered ER, PR, c-erb-B2 and p53+ when more than 10% of the cells showed specific staining, in agreement with our previous studies (3-5). In the case of p53, this degree of staining has previously been found by us to be almost invariably associated with expression of the mutant protein (6). The Ki-67 score was expressed directly as the percentage of reactive tumor cells. Ten high-power fields from a representative slide of each tumor were evaluated independently by two observers, and a consensus was reached using a teaching microscope in case of discrepancy.

Statistics. The survival of the patients in relationship to each tested parameter was calculated by means of life tables and the Kaplan-Meier method. The survival was considered from the time of initial surgery. Continuous variables were compared with each other directly by means of Pearson’s correlation test if they showed a Gaussian distribution, otherwise by means of Spearman’s test. Qualitative variables were compared with each other by means of contingency tables and the Chi-square test, or Fisher’s exact test when applicable. The statistical analysis was performed using the GraphPad Prism biomedical statistical package (GraphPad Software, Inc., San Diego, CA, USA). Values were considered significant, when $p < 0.05$.

Besides all the cited immunohistochemical determinations, tumor stage and grade, together with patient age, were also included into the statistical analysis.

Results

Of the studied tumors, 25 (55.6%) expressed mutant p53, 22 (48.9%) expressed BCL-2 and 25 (55.6%) expressed FAS. P53, BCL-2 and FAS expression were not correlated with each other.

The only tested factors associated with significantly better survival of the patients were low tumor stage ($p=0.0074$; Figure 2), the absence of metastatic regional nodes ($p=0.0009$; Figure 3) and FAS expression ($p=0.029$, Figure 4). FAS expression was not correlated with the expression of any other tested parameter.

Discussion

The correlation with survival of FAS expression determined by means of immunohistochemistry has, to our knowledge, only been studied before in laryngeal cancer by Jäckel et al. (7). They found a positive relationship between FAS expression and lymphoplasmocytic stromal reaction, but none with patient survival. However, Jäckel et al. used an antibody (APO-1/CD95) different from the one used by us (GM30/CD95), which in their case yielded a positive reaction in 87 out of their 88 studied cases. This made quantitation of each individual reaction necessary, which is always problematic when dealing with immunohistochemical results, as we know from our own experience with the technique (3-5). In our present case, we were fortunate enough that using our antibody only 55% of the studied cases were immunoreactive, and that any degree of reactivity was relevant for the statistical workup, something we also encountered in a previous study on another apoptosis-related protein, BCL-2, in breast cancer (5). Thus, our cases could be neatly divided into "FAS-positive" and "FAS-negative", and the FAS-positive ones showed a significantly longer survival than their counterparts. This could eventually make the antibody used by us (GM30/CD95) especially useful for clinical purposes, if our results are confirmed in further studies. Our present results
are very similar to the ones obtained by Chan et al. (8) in a series of similar size to ours, but of oesophageal squamous cell carcinomas, a tumor histologically identical to squamous cell laryngeal cancer. They also found FAS expression to be significantly associated with a better survival. However, Chan et al. again used another antibody, Fas C-20, different from that used by our group and that used by Jäckel et al. All these results indicate that the choice of antibody will be critical in future studies on the clinical relevance of FAS expression in human cancers.

With regard to the other apoptosis-related proteins studied by us, p53 and BCL-2, a number of previous reports have dealt with their role in laryngeal cancer. The existing data on BCL-2 are contradictory: whereas Hirvikoski et al. (9) arrived at our same conclusion, i.e., that Bcl-2 lacks any prognostic significance in laryngeal squamous cell carcinoma, two other studies tend, at least partly, to hold the opposite view. Thus, Jäckel et al. (10) found that the co-expression of p53 and BCL-2 was associated with poor survival. We tested this combination in our series and found no such association. Furthermore, Georgiou et al. (11) found that Bcl-2 expression predicted a significantly unfavourable outcome in their series, however, its small size (only 30 cases) should lead us to consider their results with some degree of caution.

On the other hand, the conclusions on the role of p53 in laryngeal cancer are rather unanimous: with the exception of the report by Jäckel et al., who found a correlation between the co-expression of p53 and BCL-2 with survival (10), the generally held view is that p53 lacks any impact on prognosis. This was confirmed by Assimakopoulos et al. (12), Teppo et al. (13) and ourselves in the present study. Although Pizem et al. (14) have reported that wild-type p53 represses survivin expression, and that a high level of survivin expression is associated with poor survival in laryngeal squamous cell cancer, p53 expression alone does not seem to correlate with prognosis in this kind of tumor, at variance with what happens in the case of many other human cancers.

**Conclusion**

FAS expression as determined by means of immunohistochemistry using the GM30/CD95 monoclonal antibody was associated with a significantly better survival in a series of squamous cell laryngeal cancer patients. On the other hand, it was not associated with the two other significant prognostic factors for survival, namely tumor stage and nodal invasion, and thus seems to be an independent predictor of prognosis in laryngeal cancer.
References


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