ORIGINAL ARTICLE

HSP-90 Expression as a Predictor of Response to Radiotherapy in Head and Neck Cancer Patients

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Abstract

Introduction and objectives: HSP-90 is an intracellular protein that protects the cell from environmental stress situations. The overexpression of HSP-90 isoforms could serve as a mechanism of resistance to radiotherapy for tumour cells. We studied this effect in a sample of head and neck tumours.

Methods: We included 87 patients diagnosed with oral cavity, oropharynx, larynx and hypopharynx tumours. We studied the expression of the HSP-90 isoforms by real-time PCR on pre-treatment biopsy samples. We analysed the relationship between HSP-90 expression levels and local relapse of the tumour with CRT decision trees.

Results: The expression levels of the inducible cytosolic isoform (HSP90AA) allowed the definition of two groups of patients with different rates of local relapse. The group with a low expression level showed a 2.9% local relapse rate, while the group with a high expression level showed a 38.2% rate.

Survival curves showed differences in time to local relapse for both groups of patients. These differences did not reach statistical significance.

Conclusions: Radiotherapy response was related to expression levels of HSP-90 in a sample of head and neck cancer patients. This result could prove useful in the selection of treatments for this group of patients.

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Introduction

Tumours of the head and neck are the fifth highest in incidence in Europe, affecting more than 100,000 people each year. In recent years major advances have been made in the so-called organ-preservation therapies, which seek to avoid mutilating surgery as the first approach in the treatment of these patients.

These treatments are principally based on the application of external radiotherapy, associated or otherwise with chemotherapy, and have different schemes. There is the possibility of surgical rescue for the patients for whom these therapies fail, although with fewer possibilities of success than the initial surgery. This results in significantly decreased survival in this group of patients.

Establishing predictive response factors to radiotherapy would enable treatment to be individualised, avoiding ineffective treatment and maximising the possibilities of controlling the disease for each patient. The expression of different proteins related to cellular response to radiotherapy has been investigated along these lines.

The Heat Shock Protein-90 (HSP-90) is a protein which is present in all cells and whose mission is to fold and form a wide range of proteins termed “client” proteins. In situations of environmental stress (hypoxia, heavy metals, acidosis) the expression of HSP-90 increases to stabilise the intracellular proteins and prevent damage to the cell.

An increased expression of HSP-90 in different types of cancers has been demonstrated. This might be explained as the cell’s attempt to maintain its homeostasis in the hypoxic and acidic environment of the tumour or as means of countering the presence of mutated proteins, the result of the genetic alterations of oncogenesis.

Our study seeks to investigate the expression of HSP-90 in a sample of head and neck tumours treated with radiotherapy, and to assess whether the over-expression of HSP-90 gives the tumour cells any advantage in response to treatment.

Methods

This study was approved by the hospital’s ethical committee and complies with the principles of the Helsinki declaration.

All the patients were assessed by a multidisciplinary oncological committee in order to select their treatment, which was determined following the centre’s protocols. A total of 87 patients were included, diagnosed in our centre with squamous carcinomas of the oral cavity, oropharynx, larynx and hypopharynx, who had undergone treatment with radiotherapy or chemo-radiotherapy, and from whom it had been possible to take a valid tumour sample. The clinical characteristics of the tumours can be seen in Table 1.

The presence of the HPV virus was studied in the samples of tumours of the oropharynx by PCR. The presence of HPV 16 was demonstrated in 3 of the 36 cases of tumours in this site.

The relevant clinical information was obtained from a database which gathered information prospectively from all the patients diagnosed and treated in our centre since 1985.

Only patients with a minimum follow-up of 2 years were considered in this study.

RNA Analysis

Prior to any treatment, biopsy samples were taken in which the levels of expression of the 3 HSP-90 isoforms were determined by PCR in real time (RT-PCR): HSP90AA (inducible
cytosolic), HSP90AB (constitutive cytosolic) and HSP90B1 (located in the endoplasmic reticulum).

Immediately after surgery the tumour biopsies were submerged in 1 ml of RNALater (Qiagen) and stored at −80 °C until processed. The total RNA was extracted using Ultraspec (Biotecx Laboratories, Inc.) following the manufacturer’s instructions. Inverse transcription was performed from 1 μg RNA with the High-Capacity cDNA Archive Kit with random hexamers (Applied Biosystems, Foster City, CA). After reverse transcription, the transcript levels of the genes studied by RT-PCR were analysed in an ABI Prism 7900HT using validated predesigned assays—TaqMan Gene Expression Assays (HSP90AA1, Hs00743767_sH; HSP90AB1, Hs00607336_gH; HSP90B1, Hs00427665_g1, Applied Biosystems) and universal amplification parameters. The relative expression of each transcript was expressed as the transcript ratio of the gene/transcript β-actin.

### Statistical Analysis

Decision trees were used (Classification and Regression Trees—CART) to detect cut-off points in the study variables. The Kaplan–Meier method was used for the actuarial calculations of survival.

### Table 1  Demographic Data of the Patients Included in the Study.

<table>
<thead>
<tr>
<th>No.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years; range)</td>
<td>61.1 38.1–86.1</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>80 92.0</td>
</tr>
<tr>
<td>Female</td>
<td>7 8.0</td>
</tr>
<tr>
<td>Site</td>
<td></td>
</tr>
<tr>
<td>Oral cavity</td>
<td>4 4.6</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>36 41.4</td>
</tr>
<tr>
<td>Larynx</td>
<td>36 41.4</td>
</tr>
<tr>
<td>Hypopharynx</td>
<td>11 12.6</td>
</tr>
<tr>
<td>Regional spread N</td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>45 51.7</td>
</tr>
<tr>
<td>N+</td>
<td>42 48.3</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>9 10.3</td>
</tr>
<tr>
<td>II</td>
<td>17 19.5</td>
</tr>
<tr>
<td>III</td>
<td>27 31.0</td>
</tr>
<tr>
<td>IV</td>
<td>34 39.0</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
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<tr>
<td>RT</td>
<td>43 49.4</td>
</tr>
<tr>
<td>CRT</td>
<td>41 47.1</td>
</tr>
<tr>
<td>BRT</td>
<td>3 3.4</td>
</tr>
<tr>
<td>Induction chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>27 31.0</td>
</tr>
<tr>
<td>No</td>
<td>60 69.0</td>
</tr>
</tbody>
</table>

BRT, bioradiotherapy: concomitant cetuximab; CRT, chemoradiotherapy (concomitant Cis-Pt).

![Figure 1](image-url)  CRT classification tree to determine a cut-off point of HSP90AA levels with respect to the possibility of local relapse.

### Results

The overall survival of the sample at 5 years was 61.4%. The adjusted survival of all the patients was 88.5%.

In the course of follow-up a total of 28 patients (32.2%) presented a local relapse of their tumour after treatment. The median time until relapse was 9.63 months (range 4, 44–38, 37 months).

A CART type decision tree model was adjusted with the patients’ clinical data and the levels of expression of the HSP-90 genes.

The isoform HSP90AA (inducible cytosolic) was the only isoform which was demonstrated to have the power to predict local relapse in our series. The algorithm identified a cut-off point which separated the sample into 2 groups with differentiated expression of HSP90AA (Fig. 1). In the group with low expression, local relapse occurred in 7/32 patients (21.9%) whereas in the group with high expression it appeared in 21/55 patients (38.2%).

The Kaplan–Meier curve analysis showed how the two subpopulations presented differences in local relapse-free time. This difference did not reach statistical significance (P=.142) (Fig. 2).

Comparisons of the groups defined by the decision tree did not show significant differences in the distribution of age, location, stage or treatment applied between both groups (Table 2).

### Discussion

The HSP proteins play an important role in maintaining cellular homeostasis. It is estimated that on its own, HSP-90 might represent 1%-2% of a cell’s total proteins. One of its numerous functions is to stabilise the cellular proteins in situations of environmental stress (heat, acidosis or oxygen free radicals). Because of the central role it plays in the regulation of numerous metabolic pathways associated
with tumour development (p53, mTOR, etc.) it has been the object of intensive research as a therapeutic target.\(^{10,11}\)

Our study seeks to relate the levels of expression of HSP-90 with response to radiotherapy. The levels of expression of the inducible cytosolic isoform HSP-90 (HSP90AA) enabled the patients to be classified into two groups with different probabilities of local relapse after radiotherapy. Thus, the patients with high expression of HSP90AA presented a probability of relapse 1.74 higher than the patients with low expression. Given the limitation of the sample size, this result did not reach statistical significance. No significant differences were found in other variables such as the location or spread of the tumour between the groups of high and of low expression of HSP-90. This appears to indicate that HSP-90 expression is a predictive factor independent of the response to radiotherapy.

The over-expression of HSP-90 in tumours of the head and neck has been interpreted as one of the baseline mechanisms of resistance to chemotherapies. Misso et al. showed how inhibition of HSP-90 causes sensitisation of the Hep-2 cells to Docetaxel,\(^{10}\) whereas Yang et al. found the same relationship with 5-FU.\(^{11}\)

Similarly, various target proteins of HSP-90 have been associated with the cell’s radio-protective response: ErbB2, Raf-1, Akt, EGFR, IGF-1R, amongst others.\(^{12}\) Furthermore, it has been demonstrated in vitro that several inhibitors of HSP-90 function sensitise the tumour cells to the action of radiotherapy.\(^{12-14}\)

According to some studies, the efficacy of HSP-90 inhibitors is directly associated with its level of activity, so that they require an over-expression of HSP-90 to demonstrate an anti-tumour effect.\(^{10}\)

Our study shows how the clinical response of tumours to radiotherapy is associated with the level of expression of HSP-90, suggesting that this association of over-expression with response is significant in vivo as well. This result would limit the use of HSP-90 inhibitors to the subgroup of patients with high expression and would help in the choice of treatment.

Because of the heterogeneity of the cases included in this study and the retrospective nature of the analysis presented, more studies are considered necessary to confirm the association between the expression of SHP-90 and response to radiotherapy. A wider analysis should help to clarify whether the use of chemotherapy or biological therapy would be influenced by the expression of HSP-90, and to confirm that this marker is valid in the different tumour sites.

### Conclusions

The clinical response to treatment with radiotherapy seems to be associated with the expression of HSP-90 in a sample of tumours of the head and neck. Generalisation of this result would require confirmation in prospective studies, but it is of great interest in the selection of treatments for this group of patients.
Funding

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Conflict of Interests

The authors have no conflict of interests to declare.

References