Comparative Analysis of Hsp10 and Hsp90 in Large Bowel Healthy Mucosa and Adenocarcinomas

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1. BACKGROUND

Heat shock proteins (Hsps) are an important class of molecules with various functions. Their classic role is to assist other proteins in folding and refolding and, when proteins are defective or irreversibly misfolded, to drive their degradation. For this reason, some Hsps are also named molecular chaperones. Despite evolution, this class of proteins has also acquired ‘extrachaperoning’ roles such as participation in immune system regulation, cell differentiation, programmed cell death and carcinogenesis. Hsp10 is a partner of Hsp60 in the Hsp60/Hsp10 folding machine, but numerous scientific studies have shown that Hsp10 may also play other roles. In fact, Hsp10 seems to have an immunomodulatory activity and a role in tumor progression. Hsp90 regulates late-stage maturation, activation and stability of a range of ‘client’ proteins, such as HER2, EGFR and B Raf, some of which are involved in signal transduction and other key pathways important for malignancy in several cancers, including large bowel carcinomas. The aim of the present study was to evaluate levels and expression of Hsp10 and Hsp90 in a series of samples of large bowel mucosa obtained from healthy controls and patients with adenocarcinomas.

2. THE CHAPERONING SYSTEM

<table>
<thead>
<tr>
<th>Heat shock protein</th>
<th>Cellular location</th>
<th>Proposed function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsp90</td>
<td>Mitochondria</td>
<td>Protein folding, prevention of protein aggregation, cell cycle arrest, modulation of programmed cell death</td>
</tr>
<tr>
<td>Hsp70</td>
<td>Cytoplasm, nucleus and membranes</td>
<td>Protein folding and protection from aggressive cytotoxic stimuli, stabilization of higher order conformation</td>
</tr>
<tr>
<td>Hsp90</td>
<td>Cytoplasm, cell membranes, ER</td>
<td>Protein folding and transport, protection from misfolding</td>
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3. METHODS

Twenty samples of large bowel human mucosa from healthy control subjects and twenty samples of large bowel adenocarcinomas with moderate grade of differentiation, were obtained from the DICHIROS Department of the University of Palermo, Italy. RT-PCR and Western Blotting Analyses were performed on these samples in order to study gene and protein expression of Hsp10 and Hsp90 (both Hsp90α and Hsp90β isoforms). Moreover, an immunohistochemical study for Hsp10 and Hsp90 was performed to evaluate the localization of these proteins in both the epithelium and the lamina propria.

4. RT-PCR ANALYSES

![RT-PCR Analysis](image)

Note: RT-PCR analyses show that mRNA expression levels of Hsp10, Hsp90α and Hsp90β are higher in samples of adenocarcinoma than normal mucosa. Quantitative RT-PCR was performed using LightCycler II Realtime Transcription (Promega) for the FAMA retro-transcription, and GoTaq Fast DNA Polymerase for amplification of cDNA.

5. WESTERN BLOTTING ANALYSES

![Western Blotting Analysis](image)

Note: Western Blotting Analyses showed that the expression of Hsp10 and Hsp90 were highest in adenocarcinoma’s samples compared to normal mucosa. The primary antibody used against Hsp10 was a rabbit polyclonal, diluted 1:500 (Gene Tech). The primary antibody used against Hsp90 was mouse monoclonal, diluted 1:500 (Gene Tech). The secondary antibody used against Hsp10 was mouse monoclonal, diluted 1:200 (SANTA CRUZ BIOTECHNOLOGY). Nuclear counterstaining was done using haematoxylin (DAKO). Bar: 100μm.

6. IMMUNOHISTOCHEMICAL ANALYSES

![Immunohistochemical Analysis](image)

Note: The evaluation of the immunohistochemical expression of Hsp10 and Hsp90 was performed on the epithelium and on the lamina propria of samples of normal mucosa and adenocarcinoma. Hsp10 levels, both in epithelium and in lamina propria, increase from normal mucosa to adenocarcinoma. Hsp10 level increases significantly in lamina propria of adenocarcinoma, compared to normal mucosa. There are no changes in the epithelium. Immunostaining was performed using Histostain-plus Kit 3rd Generation Detection Kit (Invitrogen) on 4-μm sections from each case. The primary antibody used against Hsp10 was mouse monoclonal, diluted 1:200 (SANTA CRUZ BIOTECHNOLOGY). Nuclear counterstaining was done using haematoxylin (DAKO). Bar: 100μm.

7. SUMMARY OF RESULTS

RT-PCR analysis showed a higher gene expression of Hsp10 and Hsp90 in adenocarcinoma samples compared to healthy mucosa. The Western Blotting analysis confirmed a greater amount of Hsp10 and Hsp90 proteins in the samples of adenocarcinoma of large bowel compared to healthy mucosa. Finally, levels of Hsp10 were higher in adenocarcinoma compared to normal mucosa in both the epithelium and in the lamina propria, as revealed by immunohistochemistry. By contrast, Hsp90 levels were not significantly different in the epithelium, while they were higher in the lamina propria of adenocarcinoma samples compared to normal mucosa.

8. CONCLUSION

These data suggest that Hsp10 and Hsp90 may be involved in the carcinogenesis of the large bowel by different molecular mechanisms.

9. REFERENCES


10. ACKNOWLEDGEMENTS

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