COMPARATIVE ANALYSIS OF HSP10 AND HSP90 IN LARGE BOWEL HEALTHY MUCOSA AND ADENOCARCINOMAS

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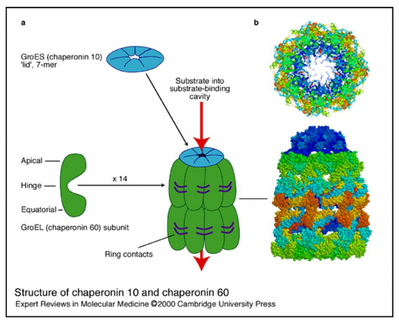
1.BACKGROUND

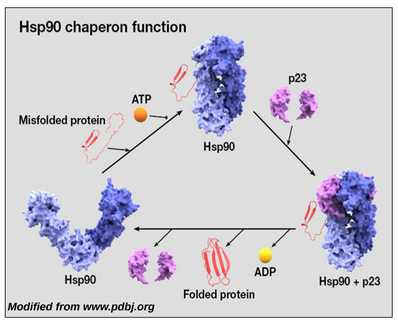
Heat shock proteins (Hsps) are an important class of molecules with various functions. Their classic role is to assist other proteins in folding and re-folding and, when proteins are defective or irreversibly misfolded, to drive their degradation. For this reason, some Hsps are also named molecular chaperones. During 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 Hsp60/10 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 BRAF, 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.

Hsp10



Note: The human Hsp10 (10000 Da) is the product of HSPE1gene located on chromosome 2 (2q33.1)

Hsp90

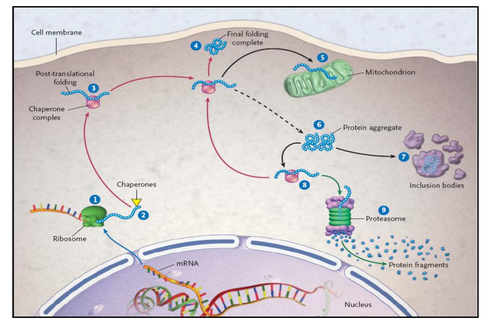


Note: The human Hsp90 (90000 Da) is present in two isoforms. Hsp90α is the product of HS90AA1 gene located on chromosome 14(14q32.33). Hsp90β is the product of HSP90B1 gene located on chromosome 12(12q24.2-q24.3).

2.THE CHAPERONING SYSTEM

Major groups	Cellular location	Proposed function
Hsp60	Mitochondria	Protein folding, prevention of protein aggregation, cytoprotection, macrophage activator possibly through Toll-like receptor.
Hsp70	Cytoplasm, mitochondria, nucleus	Protein folding and degradation, prevention of protein aggregation, cytoprotection, anti-apoptotic function.
Hsp90	Cytoplasm, cell membrane, ER	Protein folding and assembly, cytoprotection, intracellular signaling (e.g steroid receptor), cell-cycle control.
Hsp110	Cytoplasm, nucleolus	Protein folding and transport. Prevention protein aggregation.
Small Hsps (e.g., Hsp10)	Cytoplasm, mitochondria, nucleus, cell membrane, etc.	Protein stabilization and catabolism

Modified from: Haak J and Kregel KC. Novartis Foundation Symposium, 2008.



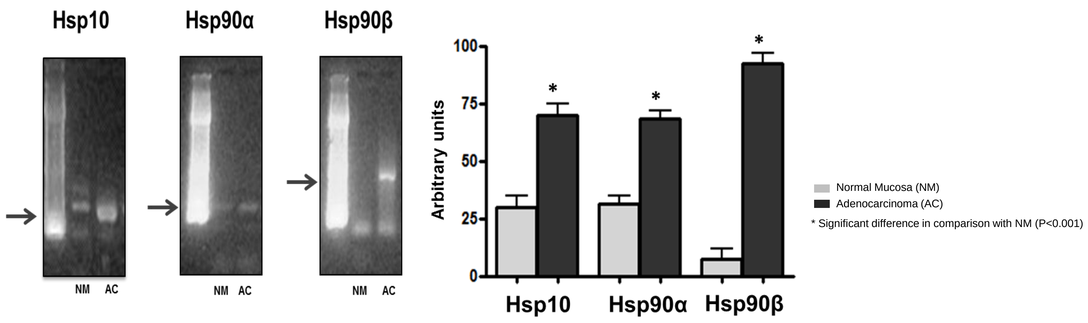
Macario AJL & Conway de Macario E, N Engl J Med 2005

Note: Chaperones, many of which are Heat shock proteins (Hsps), are a class of molecules highly conserved during evolution with essential roles in cell survival. The whole complement of Hsps-chaperones of an organism constitutes a physiological system that was called "the chaperoning system". They were initially classified according to their sequences and molecular masses. They may differ in time of expression (developmental, stress, etc.) and substrate specificity (nascent proteins, stress-damaged proteins etc).

3.METHODS

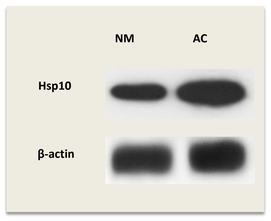
Twenty samples of large bowel human mucosa from healthy control subjects and twenty samples of large bowel adenocarcinomas with moderate grade of differentiation, were obtained from the DICHIRONS Department of the University of Palermo, Italy. RT-PCR and Western Blotting analyses were performed on these 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.

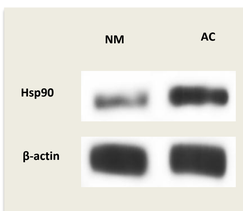
4.RT-PCR ANALYSES

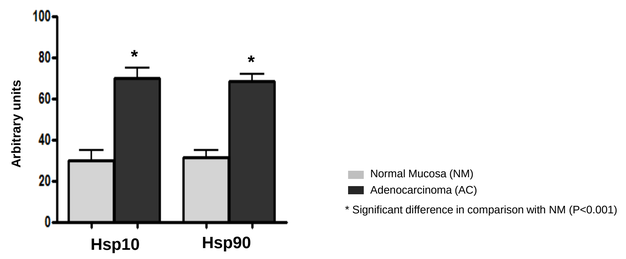


Note: RT-PCR analyses show that mRNA expression levels of Hsp10, Hsp90α and Hsp90β are higher in samples of adenocarcinoma than normal mucosa. Qualitative RT-PCR was performed using ImProm –II Reverse Transcriptase (Promega) for the RNA retro-transcription, and GoTaq Flexi DNA Polymerase for amplification of cDNA.

5.WESTERN BLOTTING ANALYSES

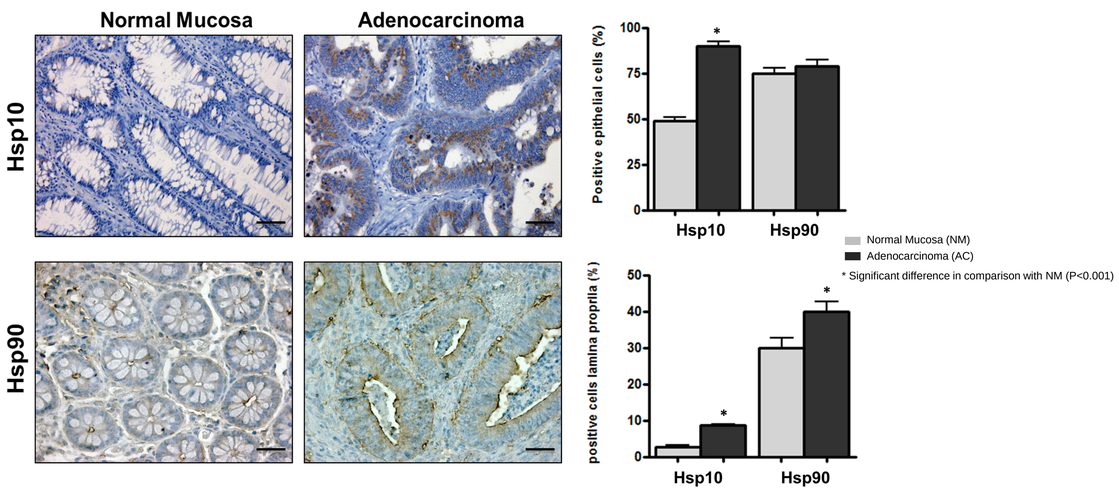






Note: Western Blotting Analysis showed that the expression of Hsp10 and Hsp90 was highest in adenocarcionoma's samples compared to normal mucosa. The primary antibody used agoinst Hsp10 was a rabbit polyclonal, diluited 1:500 (Gene Tex). The primary used against Hsp90 was mouse monoclonal, diluited 1:500 (SANTA CRUZ BIOTECHNOLOGY). The primary antibody against β actin was rabbit polyclonal, diluited 1:500 (Gene Tex). It was used like internal housekeeping.

6.IMMUNOHISTOCHEMICAL ANALYSES



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 adenocarcinomas. Hsp10 levels, both in epithelium and in lamina propria, increase from normal mucosa to adenocarcinoma. Hsp90 levels increase significantly only in lamina propria of adenocarcinoma, compared to normal mucosa. There are no changes in the epithelium. Immunostaining was performed using Histostain-plus Kit 3rd Gen IHC Detection Kit, (Invitrogen) on 4–5µm sections from each case. The primary antibody used against Hsp10 was rabbit polyclonal, diluited 1:300 (Gene Tex). The primary antibody used against Hsp90 was mouse monoclonal, diluited 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

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10. ACKNOWLEDGEMENTS

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