

# ACTIVIN A /FOLLISTATIN AND MAGE-1 AND MAGE-3 GENE TRANSCRIPTS: MARKERS FOR HEPATOCELLULAR CARCINOMA

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## Introduction

- ❖ **Hepatocellular carcinoma (HCC)** is a growing medical problem in many countries (including Egypt). The molecular mechanisms involved in HCC development still remains obscure and its prognosis is still disappointing. Because cell proliferation and apoptosis are linked during the development of HCC, so, growth and proapoptotic factors may represent useful markers for it.
- ❖ **Activins** are members of tumor growth factor  $\beta$  (TGF- $\beta$ ) supergene family of secreted proteins. There are three molecular isoforms of activins : activin A ( $\beta$ A- $\beta$ E), activin B ( $\beta$ B- $\beta$ B) and activin AB ( $\beta$ A- $\beta$ B). **Activin A** expression has been detected in various human tissues. It enhances apoptosis and inhibits cell proliferation, angiogenesis and growth of vascular endothelial cells, this supports its probable preventive role against cancer progression and metastasis.
- ❖ **Follistatin** is a secretory glycoprotein that binds activin A with high affinity and slow dissociation rate, also, it binds proteoglycans forming a cell surface barrier preventing activin A from accessing its receptor. In addition, it may accelerate endocytosis and degradation of activin A.
- ❖ **Activin A/Follistatin System:** Activin A increases the expression and secretion of follistatin in rat anterior pituitary cells. While, follistatin modulates activin A activity via a negative feedback loop. Taken together, the activin A/follistatin system may modulate growth of parenchymal liver cells.
- ❖ **The human melanoma Antigen (MAGE) genes** are switched on and up-regulated in tumors of various histological types. They are not expressed in normal cells, except in the male germ cells, placental cells and developing embryo. They have the potential to encode tumor-specific antigenic peptides which combine with the major histocompatibility complex class I molecules. Thus they can elicit a humoral and a cellular immune response in the host.

## Aim of the work

The present study aims to validate the role of serum **activin A** and **follistatin** as well as **melanoma antigen genes (MAGE-1 and MAGE-3)** transcripts in the peripheral blood and malignant liver tissues as diagnostic markers for HCC.

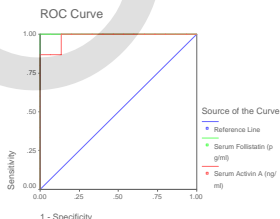
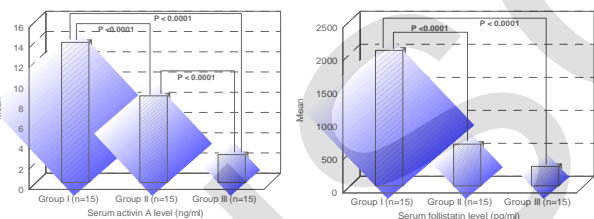
## Material and Methods

### This study included;

- ❖ **Group I:** 15 patients with HCC. **Group II:** 15 patients with liver cirrhosis complicating chronic viral hepatitis without HCC. **Group III:** 15 healthy subjects.
- ❖ **All patients and healthy subjects** were subjected to abdominal ultrasonographic examination, routine laboratory tests, hepatitis virus markers and alpha-fetoprotein (AFP). The severity of liver disease was graded as class A, B and C using numerical scoring system according to Child-Pugh classification, while the HCC stage was assessed according to the Cancer of the Liver Italian Program. Paired core liver biopsies were taken from HCCs and surrounding noncancerous liver tissues in all cirrhotic patients with HCC.
- ❖ Serum free **activin A** and **follistatin** were estimated by ELISA.
- ❖ mRNA was extracted from peripheral blood of all subjects and from HCC tissues and surrounding noncancerous liver tissues of HCC patients. The expression of **MAGE-1** and **MAGE-3** in these samples was detected by reverse transcription and concomitant PCR.
- ❖ The **sensitivity** and **specificity** of serum activin A and serum follistatin as markers for diagnosis of HCC have been determined by plotting a receiver-operating characteristic (ROC) curve and determining their cut-off values. The sensitivity and specificity of MAGE-1 and MAGE-3 expression has been calculated.

## Results

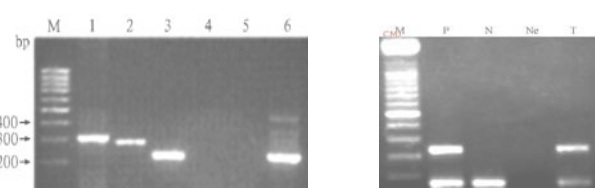
Statistical comparison between serum activin A and follistatin levels in the three studied groups.



Receiver operating characteristic curve (ROC) for serum activin A and serum follistatin.

Parameters	Cut-off value	Sensitivity	Specificity
Serum activin A (ng/ml)	9.8	100%	87.7%
Serum follistatin (pg/ml)	1050	100%	100%
MAGE-1 gene expression	-	53.3%	100%
MAGE-3 gene expression	-	40%	100%

Sensitivity and specificity of serum activin A, serum follistatin and MAGE gene expression as markers for the diagnosis of HCC.



M= 100 bp DNA ladder.

Lanes 1, 2 and 3: expression of MAGE-1, MAGE -3 and -actin genes in peripheral blood of a HCC patient. Lanes 4, 5 and 6: expression of MAGE-1, MAGE -3 and -actin genes in peripheral blood of a cirrhotic patient.

M= 100 bp DNA ladder.

Lanes 1 and 2: expression of MAGE-1 and MAGE -3 genes in HCC tissue. Lanes 3 and 4: expression of MAGE-1 and MAGE -3 genes in the non-cancerous cirrhotic tissue. Lanes 5: expression of -actin gene in HCC tissue

Statistical comparison between different studied groups as regards MAGE-1 and MAGE-3 gene expression.

Parameters	Group I (n=15)	Group II (n=15)	Group III (n=15)	$\chi^2$	P value
<b>MAGE-1 gene expression:</b>					
- Peripheral blood:					
Absent (%)	7 (46.7)	15 (100.0)	15 (100.0)	19.46	0.0006
Present (%)	8 (53.3)	0 (0.00)	0 (0.00)		
- HCC tissue:					
Absent (%)	7 (46.7)	-	-		
Present (%)	8 (53.3)				
- Noncancerous liver tissue:					
Absent (%)	15 (100.0)	-	-		
Present (%)	0 (0.00)				
<b>MAGE-3 gene expression:</b>					
- Peripheral blood:					
Absent (%)	10 (66.7)	15 (100.0)	15 (100.0)	11.25	0.003
Present (%)	5 (33.3)	0 (0.00)	0 (0.00)		
- HCC tissue:					
Absent (%)	9 (60.0)	-	-		
Present (%)	6 (40.0)				
- Noncancerous liver tissue:					
Absent (%)	15 (100.0)	-	-		
Present (%)	0 (0.00)				

## Conclusions & Recommendations

- Serum **Activin A** has a sensitivity of 100% and a specificity of 87.7% in discrimination between liver cirrhosis with and without HCC, while serum **follistatin** has a sensitivity of 100% and a specificity of 100% in detecting HCC. However, the value of both serum activin A and serum follistatin in predicting the prognosis of HCC has not been proven. Also, the superiority of both these markers over AFP cannot be evaluated in the present study because all studied HCC patients had a serum AFP above its cut-off value (200 ng/ml).
- Taken together, these results indicate that both serum activin A and follistatin have a high accuracy for diagnosis of HCC. Therefore, they can be used as useful additional indicators for screening of HCC among patients with liver cirrhosis.
- **This deregulated activin A/ follistatin axis** in patients with HCC may offer a novel option for a more selective approach in the treatment of HCC. For instance, chemical inhibitors interfering with the activin A signaling pathway may represent promising cancer therapies.
- **MAGE-1 expression** has a sensitivity of 53.3% and a specificity of 100% in the detection of HCC. However, the sensitivity of **MAGE-3 expression** is 40% and its specificity was 100% in detection of HCC. Therefore, MAGE-1 and MAGE-3 gene expression can be used as screening indicators for HCC.
- The tumor-specific expression of MAGE genes may represent a potential target for tumor-specific immunotherapy in HCC patients. For instance, the use of MAGE antigenic peptide vaccine can be considered as a promising future approach for treatment of HCC.

- ❖ **No significant correlation** was found between serum activin A and serum follistatin levels ( $r = 0.100$ ,  $P = 0.722$ ).
- ❖ **No significant relationship** between serum levels of activin A and follistatin, MAGE-1 and MAGE-3 expression on one hand and patient age, serum levels of ALT, AST, GGT, alkaline phosphatase, AFP, Child-Pugh score and tumor maximum diameter, stage and histopathological grade on the other hand ( $P > 0.05$ ).