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Original Research Article

# EXPRESSION OF ECT2 GENE IN GALLBLADDER CARCINOMA

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**ABSTRACT**

**Background:** ECT2 is a transcription regulator that is activated by various intra- and extra-cellular stimuli such as cytokines, oxidant-free radicals, ultraviolet irradiation, and bacterial or viral products. It forms homo- and heterodimers and upregulate or suppress expression of many genes that are involved in many important physiological processes, chronic inflammation and has been associated with several types of cancers. The aim of the study was to look at the expression of ECT2 in carcinoma of the gallbladder patients.
**Materials and Methods:** The study includes 39 carcinoma of the gallbladder (CAGB) patients as cases and 35 chronic cholecystitis (CC) patients as control. The expression profiling of ECT2 was done by semi-quantitative RT-PCR and immunohistochemistry. The results have been correlated with different clinical parameters.
**Results:** The RT-PCR data show frequently (70.9%) significant ($p<0.05$) up-regulated expression of ECT2 in CAGB compared with control. Similarly, immunohistochemistry data show significant up-regulation of ECT2 expression in 67.3% of cases than 19.4% of controls ($p=0.001$). Higher expression is significantly associated with all TNM stages and histological differentiation ($p<0.05$).
**Conclusion:** ECT2 expression profile may be critical regulatory factor in initiation, progression of CAGB patients. Since there is significantly higher expression with advanced stage, nodal involvement and distant metastasis, ECT2 may become a potential therapeutic target for the treatment of CAGB.
**Keywords:** Carcinoma of the Gallbladder, ECT2, RT-PCR, Immunohistochemistry, Gene expression.

## INTRODUCTION

Carcinoma of the gallbladder (CAGB) is the sixth most common cancer of gastrointestinal system in world and third in India.[1] CAGB has a particularly high incidence in Chile, Japan, and northern India.[2] The incidence of the disease is 2-6 folds more in women than in men.[3] It is a late presenting disease with poor prognosis. CAGB develops over 5 to 15 years when metaplasia progresses through dysplasia to carcinoma in situ and then invasive cancer.[4] While the development of CAGB has been linked to various environmental factors and critical molecular genetic events, the genetic basis is still poorly understood.[1,5] The elucidation of underlying genetic events opens the option for development of novel therapeutic targets and biomarkers.

Since the discovery of ECT2 more than 30 years ago it has served as a paradigm for inducible transcription factors because of its broad physiological and medical effects which has gained an enormous research interest.[6] ECT2 is a group of transcription regulators that forms homo- and heterodimers and up-regulate or suppress expression of many genes which are involved in critical

physiological processes, chronic inflammation and associated with several types of cancer.[7]

ECT2 mediates a crosstalk between chronic inflammation and cancer at multiple levels [8]. In chronic inflammation conditions with elevated ECT2 activity the accumulation of pro-inflammatory cytokines at the tumor site directly contributes to the pro-tumorigenic microenvironment. Activation of ECT2 in inflammatory cells in response to infectious agents like bacteria and virus, inflammatory cytokines, chemokines and proteins such as TNF-α IL-1, IL-6, IL-8, PGE2 and products released by necrotic cells lead to the production of secreted factors that enhance the growth, survival and vascularization of carcinoma cells.[8]

ECT2 directly and indirectly controls inflammation, cancer cell proliferation and survival, epithelial-to-mesenchymal transition (EMT), invasive behavior, angiogenesis and metastasis, as well as genetic and epigenetic alterations, cancer stem cell formation, cellular metabolism and therapy resistance.[9- 11]

Currently, many studies indicated that ECT2 was up-regulated in cancers including colorectal, breast and hepatocellular, lung, gastric and pancreatic cancer. However, the role of ECT2 in gallbladder carcinogenesis has not been studied so far to the best of our knowledge. We have investigated the expression of ECT2 in gallbladder cancer patients and correlated it with clinic-pathological parameters.

## MATERIALS AND METHODS

**Study Design**

This study was conducted to evaluate the expression of ECT2 in CAGB patients and to correlate its expression with various clinico-pathological characteristics by reverse transcriptase polymerase chain reaction (RT-PCR) and immunohistochemistry (IHC).

**Setting & Subjects**

The study was carried out in single surgical unit of Department of General Surgery in collaboration with Department of Pathology, Institute of Medical Sciences, and Department of Molecular and Human Genetics, Institute of Science, Banaras Hindu University, Varanasi from June 2018 to October 2020 after official approval from the ethical committee, faculty of medicine, IMS BHU. Newly diagnosed and histopathologically proven cases of CAGB as cases and chronic cholecystitis (CC) as control were included in the study. Tissue biopsies of 39 CAGB patients and 35 CC patients were collected in formalin and RNA later (Ambion, USA) for IHC and RNA purposes, respectively. The patients who received chemotherapy or radiotherapy preoperatively were excluded.

**Statistical Analysis**

The statistical analysis was done using statistical software SPSS for windows (Version 17). Chi-square test was used for non-parametric variables. Student's t-test was used for comparing two groups and one-way ANOVA test followed by Bonferroni multiple comparison post hoc test was perform for comparisons between more than two variables. P-value of less than 0.05 was considered as significant.

## RESULTS

**Demographics**

We included 39 cases of CAGB and 35 control of CC in this study. Most of the patients in our study were females 78.2 % in CAGB and 78% in CC group. Out of the 55 cases of CAGB, Early T stage (T1/T2) was present in 14 (25.5%) and late T stage (T3/T4) present in 41 (74.5%) of CAGB cases. In case of nodal status, 12 (21.8%) patients had N0, 43 (78.2%) patients had N1. Metastatic disease was present in only 3 (5.5%) patients.

**ECT2 protein expression through IHC**

Immunohistochemical staining for ECT2 expression is presented in Table 1 in both CAGB and CC groups. The expression of ECT2 was significantly higher ($p=0.001$) in the CAGB group (67.3 %) in comparison to control group (19.4%). ECT2 showed moderate immunohistochemical staining intensity in 61.8 % of CAGB cases and weak staining in 69.4 % of control group, thus the difference was significant ($p=0.001$). On comparison with different clinical parameters, ECT2 expression was found to be significantly associated with advanced stage (T3/T4), nodal metastasis, higher grade of tumor differentiation, perineural invasion ($p<0.001$) and lymphovascular invasion ($p=0.023$). However, we did not find significant association of ECT2 expression with distant metastasis ($p=0.543$).

**ECT2 expression through RT-PCR**

The integrated band density value of ECT2 mRNA expression to β-actin in CAGB was calculated by comparing the average value ± 2×SD of the normal to the tumor samples. The mean level of ECT2 mRNA in the CAGB group (0.83±0.285) was significantly higher than the control (0.41±0.136; $p<0.001$). RT-PCR showed significantly up-regulated ECT2 expression in 70.9% of CAGB compared with control group ($p<0.05$). ECT2 expression was significantly higher in advanced stage (T3/T4) in comparison to early stage (T1/T2) of the tumor. We have observed up-regulated ECT2 expression in high grade of histological differentiation. The expression ECT2 was found to be significantly associated with nodal metastasis, distant metastasis, lymphovascular and perineural invasion ($p<0.001$).

## DISCUSSIONS

Signalling pathway of ECT2, a transcription regulator. where it can regulate the expression of specific genes typically involved in immune and inflammatory responses and in cell growth.

Moreover, deregulation of ECT2 has been found in various types of human cancer.[12]

In the present study, we have hypothesized that deregulation of ECT2 and its transcriptional up-regulation and activation by post-translational modification may have critical role in gallbladder carcinogenesis. The expression profile of ECT2 can be a useful diagnostic marker for CAGB and may be a new molecular therapeutic target for carcinoma of the gallbladder. We have demonstrated that ECT2 is up-regulated at both transcript and protein levels in CAGB cases than in chronic cholecystitis ($p = 0.001$).

Gonzalez et al (2018) have reported statistically significant expression of ECT2 as moderate and strong staining intensity in colorectal carcinoma in comparison to adjacent control and non colorectal carcinoma tissue ($p<0.001$).[13] Similarly, we have also observed moderate intensity of ECT2 staining in gallbladder cancer cases as compared to controls. Sincec chronic cholecystitis is a chronic inflammatory condition, we found expression of ECT2 in chronic cholecystitis. A recent study has also reported higher ECT2 expression in colorectal carcinoma in relation to advanced T stage (T1/T2 Vs T3/T4), nodal metastasis and higher grade of differentiation ($p<0.05$).[13] The similar high expression of ECT2 in immunohistochemistry analysis were observed in our study with respect to advance stage, nodal metastasis and higher grade of differentiation($p<0.001$).

A study on breast cancer also demonstrated higher expression of ECT2 in cancer patients (71.9%) than control group. Similar to our results, they found that ECT2 expression was significantly correlated with high grade and large tumor size. However, no significant association was found between ECT2 and nodal metastasis.[14]

In our study, we demonstrate frequent up-regulation (70.9% of carcinoma of the gallbladder cases) of ECT2 mRNA expression.

Our data is consistent with the hypothesis that ECT2 is frequently up-regulated in carcinoma of the gallbladder. Since a broad spectrum of signaling pathways are regulated by ECT2 and are associated with different clinicopathological parameters, it may be used as an attractive biological target for developing new therapeutic intervention.

## CONCLUSION

In conclusion, this study showed up-regulation of ECT2 in carcinoma of the gallbladder. It may be a critical regulatory factor in initiation and progression of this cancer. Our data demonstrate frequent significantly higher expression of ECT2 with advanced stage, nodal involvement and distant metastasis. Therefore, ECT2 may become a potential therapeutic target for the treatment of carcinoma gallbladder.

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