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review

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A genetic model for gallbladder carcinogenesis and its dissemination

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Gallbladder cancer, although regarded as the most common malignancy of the biliary tract, continues to be associated with a dismal overall survival even in the present day. While complete surgical removal of the tumour offers a good chance of cure, only a fraction of the patients are amenable to curative surgery owing to their delayed presentation. Moreover, the current contribution of adjuvant therapies towards prolonging survival is marginal, at best. Thus, understanding the biology of the disease will not only enable a better appreciation of the pathways of progression but also facilitate the development of an accurate genetic model for gallbladder carcinogenesis and dissemination. This review provides an updated, evidence-based model of the pathways of carcinogenesis in gallbladder cancer and its dissemination. The model proposed could serve as the scaffolding for elucidation of the molecular mechanisms involved in gallbladder carcinogenesis. A better understanding of the pathways involved in gallbladder tumorigenesis will serve to identify patients at risk for the cancer (and who thus could be offered prophylactic cholecystectomy) as well as aid oncologists in planning the most suitable treatment for a particular patient, thereby setting us on the vanguard of transforming the current treatment paradigm for gallbladder cancer.

Key words: gallbladder cancer, genetic alteration, carcinogenesis, dissemination, biology, therapeutics

introduction

Gallbladder cancer is relatively uncommon worldwide with age-standardized incidence rates of 2/100 000 [1]. Besides, recent reports have suggested a decline in its incidence in different parts of the world [2, 3]. However, in Chile and India, gallbladder cancer remains a major problem [4, 5] (age-standardized rates from 3.9 to 8.6/100 000 [6]) with the vast majority of patients presenting with advanced disease [7, 8]. The uncommonness of gallbladder cancer has contributed to the generally poor understanding of the disease [9, 10]. However, despite the suggested declining trend of this cancer in the world, it is imperative that a better understanding of the disease and the factors influencing its course is needed to develop treatment strategies aimed at improving its overall outcome.

The most important strategy to successfully plan treatment options for gallbladder cancer is to first understand the pathogenesis of the disease. A useful tool to this end is the development of a comprehensive model of carcinogenesis akin to the Fearon–Vogelstein model for colorectal tumorigenesis [11]. By developing such a model (that will incorporate stages before the formation of an invasive cancer and up until tumour dissemination), it will be possible to lay the ground-work for a more dedicated thrust towards evidence-based, targeted initiatives in the management of gallbladder cancer. This review provides a road-map towards the development of such a model.

can we equate gallbladder cancer with colorectal cancer in terms of a natural evolution of carcinogenesis?

To even consider embarking on a tumorigenesis model, it is important to first ascertain if gallbladder cancer development progresses from benign to malignant in a step-wise evolution similar to colorectal cancer (adenoma to carcinoma).

The work by Laitio [12, 13] provided a basis for the understanding of the stages in the pathogenesis of gallbladder cancer that would eventually lead to the elucidation of a step-wise progression. Laitio [13] demonstrated that metaplasia in the gallbladder wall could develop into dysplasia which could play a significant role in gallbladder carcinogenesis [12]. The earliest work hinting at the existence of a step-wise evolution of gallbladder cancer by Albores-Saavedra et al. [14] suggested that hyperplasia could potentially develop into atypical hyperplasia and from there on to *in situ* and finally invasive cancer. The quest for a natural evolution in gallbladder carcinogenesis was further placed on a firm footing by the work of Roa et al. [15] who not only suggested the dysplasia–carcinoma sequence but also indicated that the time to transformation from dysplasia to

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advanced carcinoma was ~15 years. Roa et al. [16] went on to eloquently demonstrate the presence of metaplasia, dysplasia and *in situ* carcinoma in the vicinity of the invasive carcinoma lending further support to the metaplasia–dysplasia–carcinoma cascade first postulated by Yamagiwa and Tomiyama [17].

Duarte et al. [18] on extensive histological analysis of gallbladder benign and malignant specimens noted a significant association between intestinal metaplasia, hyperplasia and dysplasia.

The above work, however, largely originated from South and Central America [19]. In these countries, as is the case in India and most parts of the world, cholelithiasis is regarded as a cause, if not a co-factor, in the development of cancer [5]. Moreover, the p53 mutation occurs as an early event [20, 21] in these regions and is associated with gallstone disease.

An appreciation of yet another distinct pathway of disease progression was put forth by Kozuka et al. [22] who noted the presence of large adenomas (>12 mm) in relation to gallbladder cancer. This was later followed by the work of Watanabe et al. [23] suggesting three distinct pathways in gallbladder carcinogenesis—depending on the predominance of K-ras or p53 mutations.

According to Nakajo et al. [24], adenomas of either the metaplastic type or non-metaplastic type could progress to adenocarcinomas.

It thus emerged that there existed two pathways in the pathogenesis of gallbladder cancer, viz: the dysplasia–carcinoma sequence in patients with gallstones; and the adenoma–carcinoma cascade [25]. However, of the two, the more plausible cascade remains the dysplasia–carcinoma sequence [26] owing to the generally low incidence of adenomas of the gallbladder and their co-existence in the vicinity of early or advanced cancer [26] as well as some evidence to suggest different pathways being involved in adenoma development when compared with adenocarcinoma [27].

In Japan and the Far East, a morphological biliary anomaly termed anomalous pancreaticobiliary ductal junction (APBDJ) is associated with gallbladder development in a much higher frequency when compared with the rest of the world [28–30]. In APBDJ, hyperplasia is noted in up to 61% [25] of individuals. It has been postulated that hyperplasia is more likely to develop into an invasive cancer through the dysplasia–carcinoma cascade.

Thus, it is certainly possible to develop a multi-step evolutionary sequence from normal mucosa to malignant change in gallbladder cancer just as was done by Fearon and Vogelstein [11] for colorectal cancer. Only the dominant dysplasia–carcinoma tumorigenesis sequence will be discussed further.

the multi-step pathogenesis of gallbladder carcinogenesis (dysplasia to carcinoma)

In 1999, Wistuba and Albores-Saavedra [31] provided the first image of the dysplasia–carcinoma cascade based on sequential histopathological and molecular changes in the pathogenesis of gallbladder carcinoma associated with gallstones and inflammation. In 2004, Wistuba and Gazdar [32] improved on this by providing the median ages at diagnosis of each histopathological change. Figure 1 provides a diagrammatic representation of the Wistuba and Gazdar multi-step pathogenetic sequence.

While this marked the first representation of the cascade, one of the lacunae was the omission of stage of metaplasia. The importance of the metaplastic change cannot be undermined in gallbladder cancer, especially considering that >80% are

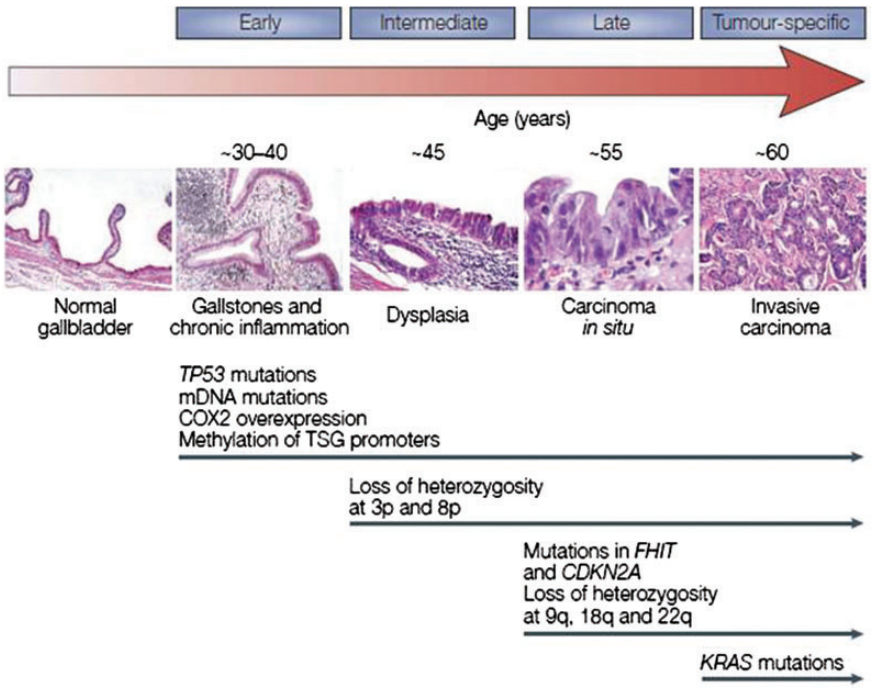


Figure 1. Multi-step pathogenesis of gallbladder cancer from gallstones proposed by Wistuba and Gazdar's [32]. TSG, Tumour suppressor gene; FHIT, fragile histidine triad; mDNA, mithochondrial deoxyribonucleic acid; COX, cycloxygenase.

adenocarcinomas. This is ironic when one considers that the overriding putative inciting factor is chronic irritation by gallstones [5]. Mere irritation should have resulted in a higher proportion of squamous differentiations rather than adenocarcinoma. More recently, Castillo et al. [33] provided an update of the genetic alterations in gallbladder cancer on the template of the 'metaplasia–dysplasia–carcinoma' sequence first postulated by Yamagiwa and Tomiyama [17].

where do we go from here?

The two cascades in the development of gallbladder cancer proposed have been based on laboratory evidence of some markers of genetic and epigenetic alterations. However, although they form the basis for any further development of the tumourigenesis model, they remain a work in progress.

Two prime alterations that need to be incorporated into the existing models are:

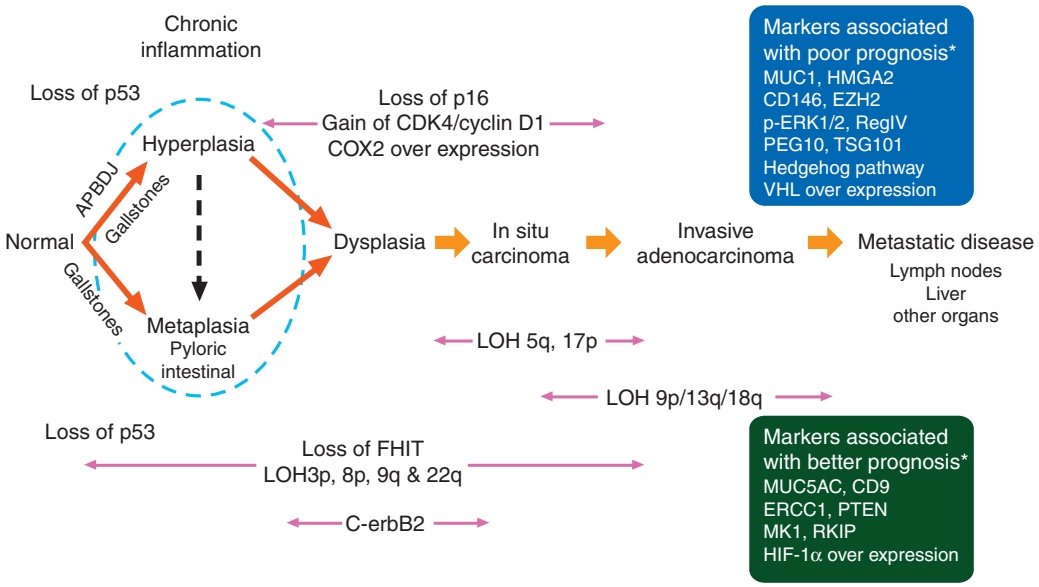
(i) Course of the disease after the development of invasive carcinoma within the gallbladder, namely tumour dissemination to lymph nodes, liver and other organs.
(ii) Updating the existing chart with markers that may be specific to the precancerous stage, invasive stage or stage of dissemination—markers which could be used in screening, diagnosis, guide treatment—choice of therapy or response to therapy.

This will permit the development of a 'working' tumorigenesis model that will enable us to better understand the disease in its entirety, thereby serving as a blue-print for planning screening/surveillance and management strategies.

Based on these principles, an updated model is presented below—'The Gallbladder Carcinogenesis and Dissemination Model' based on a review of literature with an aim to update the pre-existing cascades [25, 32, 33].

the 'gallbladder carcinogenesis and dissemination model'

The skeletal framework of this model is based on evidence indicating that primal to the development of gallbladder cancer is the existence of chronic inflammation (Figure 2) [34]. Chronic inflammation results either due to gallstones or due to changes in the bile owing to the reflux of pancreatic juice into the common bile duct induced by APBDJ [35, 36]. Chronic inflammation secondary to gallstones is more likely to lead to metaplasia (intestinal or pseudopyloric) [18] rather than hyperplasia [14], while the cholecystitis due to APBDJ on the other hand is more likely to lead to hyperplasia [37]. Further, dysplasia has been noted to develop in metaplastic epithelium [13] as well as from hyperplastic epithelium that has progressed to the atypical type [14]. It is likely that hyperplasia (that has developed at an early onset in patients with APBDJ) could also progress to



* For the exception of MUC1 & MUC5AC, the other markers are based on single studies and merit further validation

Figure 2. The 'Gallbladder carcinogenesis and dissemination model'. The proposed model takes into consideration each and every pathological change occurring in the gallbladder epithelium progressing sequentially from normal epithelial mucosa to the development of cancer via the two most common pathways, namely metaplasia/hyperplasia as well as dysplasia, and beyond the localized disease in the gallbladder to even include the spread of the cancer to regional and distant organs. The putative molecular alterations playing a role in each step are also highlighted. APBDJ, anomalous pancreaticobiliary ductal junction; FHIT, fragile histidine trait; LOH, loss of heterozygosity; HMGA2, high mobility group protein A2; CD9, mobility related protein 1; CD146, melanoma cell adhesion molecule; ERCC-1, excision repair cross-complementing group 1; EZH2, histone-lysine *N*-methyltransferase; PTEN, phosphatase and tensin homologue; pERK1/2 , extracellular signal-regulated kinase; PI3-K, phosphatidyl inositol-3 kinase; RKIP, Raf-1 kinase inhibitory protein; RegIV, member of regenerating gene family; MK-1, type 1 transmembrane protein (Ep-CAM); PEG10, retrotransposon-derived protein 10; TSG101, tumour susceptibility gene 101; VHL, Von Hippel–Lindau gene; HIF1α, hypoxia-inducible factor 1 α; CD, cluster of differentiation; COX, cycloxygenase.

dysplasia via the stage of metaplasia [25]. However, this is simply an assumption based on the significant co-occurrence of metaplasia and hyperplasia [18] that remains as yet unconfirmed. The postulate that dysplasia is a premalignant lesion that potentially develops into carcinoma *in situ* and invasive adenocarcinoma is derived from two important aspects [16], namely the consistent finding of dysplasia in the vicinity of gallbladder cancer more frequently than in non-malignant tissue on histological examination (88% versus 34%) [25, 38] and the appreciation of similar genetic alterations in dysplastic and malignant gallbladder tissue [31, 39]. After the development of invasive carcinoma of the gallbladder, the two most common sites for metastases are the lymph nodes and the liver [40].

Important to note in the above model is the non-inclusion of the adenoma–carcinoma cascade. This does not imply that the authors do not accept the existence of this cascade but rather opted to focus on the more common pathway involved in gallbladder carcinogenesis based on evidence from world literature [41].

genetic alterations in the development and dissemination of gallbladder cancer

p53

The tumour suppressor gene p53 has been found to occur in patients with gallbladder cancer throughout the world [20, 21, 42], although differences in the mutational spectra have been reported in tumour specimens from Japan and Chile (Figure 3) [43]. The most common acquired mutations are in exons 5 and 8. p53 mutations constitute one of the earliest changes in the development of gallbladder cancer—being detected in one-third of normal and dysplastic epithelia obtained from gallbladders with gallstones but without cancer [44]. Wistuba et al. [45] noted that the loss of heterozygosity (LOH) of p53 occurred earlier, and more frequently, than protein over-expression.

p16/cyclin d1/CDK4

In a recent study, Feng et al. [46] addressed the role of the p16/cyclin D1/CDK4 pathway in gallbladder cancer via the hyperplasia pathway. They noted that while expressions of CDK4 and cyclin D1 increased along with the progression of gallbladder mucosa hyperplasia with the highest expression noted in the cancer group, p16 decreased to the lowest level in gallbladder cancer. This loss of expression has been summarized by Goldin and Roa [26] to be either due to deletions in region 9p21, inactivation of the gene, LOH or by methylation.

KRAS

The evidence available is confounding with studies suggesting little or no role of the oncogene KRAS in gallbladder cancers [27, 45, 47, 48], one study reporting the detection of KRAS mutations in 59% of cancers and 73% of gallstone-induced dysplasia, while others indicating the presence of the mutation in a proportion of cancers arising secondary to APBDJ [49, 50]. The study by Itoi

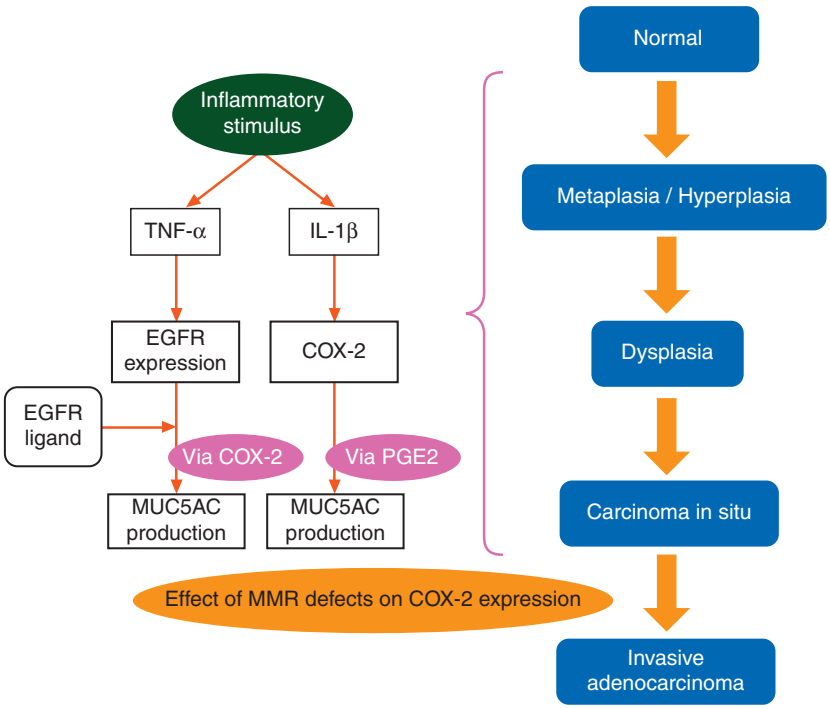


Figure 3. Inflammatory cascade hypothesis in relation to the development of gallbladder cancer. This is as yet a hypothetical model for gallbladder carcinogenesis via inflammation and inflammatory markers derived from observations in studies examining the expression of these markers in the various stages of gallbladder carcinogenesis. The model is built deriving inferences from observations in colon cancer and gastric and airway epithelial cells and cell cultures analysing the impact of inflammatory markers. TNF-α, tumour necrosis factor-α; EGFR, epidermal growth factor receptor; COX-2, cycloxygenase-2; PGE-2, prostaglandin E2; IL-1β, interleukin-1β; MMR, mismatch repair [95, 112–114].

et al. [51] analysing the status of KRAS mutations in gallbladder cancer suggests that the dominant pathway for gallbladder cancer pathogenesis may not involve KRAS mutations.

cycloxygenase-2

Cycloxygenase-2 (COX-2) over-expression occurs early in the pathogenetic cascade of gallbladder carcinogenesis being detected in high proportions in dysplasia and invasive carcinoma when compared with normal epithelium [52]. COX-2 expression has actually been noted to be reduced in adenocarcinoma tissue (59.2%) when compared with dysplastic epithelium (70.3%). COX-2 expression has also been noted to be significantly higher in epithelial hyperplasia secondary to APBDJ when compared with normal epithelium [53]. In malignant tissue, the expression of COX-2 is significantly lower in the histopathologically normal surrounding epithelium [54].

deleted in colorectal carcinoma: 18q21

LOH has been noted at deleted in colorectal carcinoma (DCC) in gallbladder cancers [45, 55] and recognized to be an early event in carcinogenesis [45]. Genome-wide association studies have recently demonstrated that a specific DCC haplotype is associated with increased susceptibility to gallbladder cancer in India, irrespective of associated risk factors [56]. A similar study reported single-nucleotide polymorphisms in DCC associated with gallbladder cancer in a Japanese population [57].

fragile histidine triad gene

Wistuba et al. [58] studied the frequency of loss and LOH at fragile histidine triad (FHIT) in normal, dysplastic and malignant tissue. They noted that while occasional FHIT abnormalities were occasionally demonstrated in histologically normal epithelium, the reduction or absence of FHIT immunostaining significantly reduced with evidence of disease progression through dysplasia and to adenocarcinoma. They noted a high correlation between immunostaining in the specimen and allelic loss. This finding of a reduction in expression of FHIT in gallbladder cancer was also noted by Koda et al. [59].

LOH of other chromosomes

As per Knudson's hypothesis [60], LOH at polymorphic loci is recognized as a hallmark of a tumour suppressor gene whose other allele is inactivated by point mutations or by some other mechanism [61]. Chang et al. [62] studied LOH on chromosomal regions 3p, 5q, 8p, 9p, 13q, 17p and 18q in gallbladder specimens with dysplasia and carcinoma. They noted that while LOH on 5q was an early change of carcinogenesis of the gallbladder, LOH on 3p and 9p was related to the progression of gallbladder carcinoma with LOH on 13q and 18q likely to be late events. They also noted that LOH on 17p occurred not only in dysplasia but also increased during the subsequent stages. On the other hand, Wistuba et al. [61] noted that LOH was an early phenomenon in the development of cancer since they found increasing proportions of LOH on chromosomes 3p, 8p, 9q and 22q in normal epithelium, dysplastic and malignant tissue.

microsatellite instability

Mismatch repair (MMR) gene mutations [63, 64] and the resultant microsatellite instability (MSI) are infrequently detected in gallbladder cancer [55, 62, 65]. An interesting perspective on this has been presented by Matsuda [66] who suggested that the association of MSI-positive tumours harbouring a favourable prognosis may imply a similarity to colon cancer. He further pointed out that the difference in the two cancers in terms of carcinogenesis may lie in the fact that gallbladder cancers tended to be MSI-L, while colon cancers were more likely to be MSI-H.

c-erb b2/HER2

The HER2 protein expression was recently studied in normal, metaplastic and invasive adenocarcinoma tissue, as well as in samples of carcinoma *in situ* [67]. Interestingly, while normal epithelium failed to show any HER2 immunoreactivity, maximal immunoreactivity was noted in metaplastic tissue (intestinal) and in tissue from carcinoma *in situ*. Once again the immmunoreactivity dropped in invasive cancer. The findings of Toledo et al. [67] are interesting as before this, Kamel et al. [68] and Kim et al. [69] had failed to demonstrate HER2 expression in dysplastic tissue while demonstrating a similar expression in malignant tissue. Chaube et al. [70] found that the expression levels for HER2 varied depending on the grade of the tumour—with decreasing expression correlating with advancing grade.

markers studied in normal, premalignant and malignant tissues of gallbladder adenocarcinoma

epidermal growth factor receptor

Studies examining the expression of epidermal growth factor receptor (EGFR) in gallbladder cancer have indicated a highly variable expression of the receptor (Table 1 [71–89]). The expression ranged from 11.3% to 100% [75, 77, 78, 80–82, 85]. The major impediment to the understanding of the role of EGFR in gallbladder carcinogenesis has been the lack of studies examining the expression profile simultaneously in the premalignant tissue samples. Kim et al. [90] noted a reduced EGFR expression in gallstone patients when compared with normal controls. EGFR and Her2 belong to a family of receptor tyrosine kinases that are anchored in the cytoplasmic membrane and share a similar structure [91]. The expression profile of Her2, as noted above, is not uniform throughout the process of gallbladder carcinogenesis. Thus, future studies in which a comparative expression profile of the expression of EGFR from normal gallbladder tissue to metaplasia/hyperplasia to dysplasia and further to *in situ* and invasive adenocarcinoma would aid in our understanding of the role of EGFR in gallbladder cancer.

MUC

Mucins are major components of the mucous viscous gel lining epithelial tissue surfaces [92]. Xiong et al. [87] reported that MUC staining on immunohistochemistry was noted mainly in the cytoplasm and/or the cell membrane rather than the nucleus. Of the nine distinct epithelial mucin genes identified in gallbladder diseases, MUC1, MUC5AC and MUC6 are normally expressed in

Table 1. Studies investigating novel alterations in normal, premalignant and malignant gallbladder cancer [71–88]

Marker	Normal	Hyperplasia	Metaplasia	Dysplasia	Carcinoma *in situ*	Invasive cancer	References
EGFR	—	—	—	—	—	38.4% (strong)	[75]
	—	—	—	—	—	83% (overall)	[85]
						33% (strong)	
	—	—	—	100% (moderate)	—	100% (strong)	[78]
	—	—	—	—	—	12.4% (strong)	[81]
	—	—	—	—	—	93.7% (overall)	[77]
						75% (strong)	
	—	—	—	—	16.6% (strong)	11.4% (strong)	[80]
	—	—	—	—	—	11.3% (strong)	[82]
CK 7	100%	—	100%	100%	—	87%	[72]
	100%	100%	100%	—	—	100%	[71][a]
	—	—	—	—	—	69.50%	[76]
	—	—	—	—	—	82%	[74]
CK20	0%	–	17%	31%	—	18%	[72]
	0%	100%	100%	—	—	100%	[71][a]
	—	—	—	—	—	28.50%	[76]
	—	—	—	—	—	27%	[74]
MUC1	0%	—	0%	35%	—	75%	[72]
	—	—	—	21.70%	—	57.40%	[87]
	—	—	—	50%	—	80%	[88]
	0–20%	—	—	—	—	78–89%	[89]
MUC2	0%	—	29%	9%	—	11%	[72]
	—	—	—	75%	100%	58%	[84]
	—	—	—	75%	—	64%	[88]
	—	—	91.70%	—	—	—	[83]
MUC5AC	89%	—	92%	53%	—	38%	[72]
	21.70%	—	—	60.80%	—	51.90%	[87]
	—	—	—	85%	90%	78%	[84]
MUC6	100%	—	100%	65%	—	27%	[72]
	—	—	—	80%	90%	91%	[84]
CDX2	—	—	—	—	—	29.20%	[73]
	—	—	91.70%	—	—	—	[83]
	0%	—	—	—	—	36.80%	[86]
	—	100%	—	—	—	45.40%	[79]

[a]Individual break-down of the cases was not provided.
EGFR, epidermal growth factor receptor; CK, cytokeratin; MUC, mucin; Strong => ≥2+.

gastric mucosa, while MUC2 is expressed in intestinal mucosa [93]. Thus, an extrapolation of this mucin expression profile in gastric cancer [93] would lead to the assumption that in gallbladder cancer developing from pyloric metaplasia, MUC1, MUC5AC and MUC6 would exhibit a rather similar expression profile, while MUC2 over-expression would predominate in cancers developing from intestinal metaplasia. However, contrary to this, the expression of MUC in gallbladder cancers is not uniform. MUC1 levels are significantly increased in gallbladder cancer, MUC2 and MUC5AC expression levels are reduced in gallbladder cancer when compared with dysplastic tissue as well carcinoma *in situ*, while the findings in the case of the two studies reporting expression levels of MUC6 are contradictory [72, 83, 84, 87–89]. The localization of MUC1 on chromosome locus 1q21 and MUC2, MUC5AC and MUC6 on chromosome locus 11p15.5 [94] may be a potential reason for this differential expression.

Finzi et al. [95] have suggested that the MUC5AC is overproduced in gallstone disease by an inflammation-dependent EGFR cascade. Vilkin et al. [96] noted that the extent of MUC5AC expression as a result of inflammation was more in pigment gallstones.

Xiong et al. [87] found that the expression of MUC1 and MUC5AC were inversely related when correlated with the extent of disease. Lower MUC1 and higher MUC 5AC expression levels were noted in tumours that were <2 cm, with no lymph node or regional tissue involvement. Also, the expression levels of MUC1 were significantly higher in tumour when compared with peritumoural tissue, while the converse was true in the case of MUC5AC. Chang et al. [72], too, appreciated the correlation of MUC1 with more aggressive tumours, while Ghosh et al. [72, 83, 84, 87–89] noted that its depolarized expression was a marker of invasion.

The above findings of the expression levels of MUC5AC in gallbladder cancer are exactly the opposite as reported in cholangiocarcinoma. Boonla et al. [97] and Park et al. [98] noted that in cholangiocarcinoma, serum MUC5AC levels were predictive of poor outcomes.

cytokeratins

Cytokeratins have been explored as markers for detecting micrometastatic disease in lymph nodes previously reported as normal on histopathology [99–101]. Micrometastatic disease is an indicator of poorer outcomes [100].

other markers

Recently, there have been numerous publications investigating markers, previously shown to be involved in other solid organ cancers, in gallbladder adenocarcinomas. Table 2 provides an overview of these studies [102–111]. These markers need validation in larger series to confirm their usefulness as prognostic markers.

the inflammatory cascade and marker expression

An interesting finding in gallbladder cancer, as noted above, is the elevation of inflammatory cascade markers such as EGFR, MUC5AC and COX-2, early in gallbladder carcinogenesis up until the stages of dysplasia and even *in situ* carcinoma followed by a reduction in their expression in invasive adenocarcinoma. This observation raises an important question—if inflammation does play a role in the development of gallbladder cancer from gallstones, then why do the levels of these markers, which are important in inflammation, undergo a reduction between the stages of dysplasia to invasive adenocarcinoma? Could these markers be actually exerting a protective influence (via the production of protective mucin through MUC5AC), while the overcoming/overwhelming of these protective forces results in invasive cancer?

Figure 3, based on the work in colon cancer and gastric epithelial cells [112] and human airway epithelial cells and cell cultures [113, 114], lends support to the first portion of the hypothesis, namely inflammation and inflammatory markers in gallbladder cancer (tumour necrosis factor-α/TNF-α and interleukin-1β/IL-1β [115]) leading to the constitutive expression of protective mucin—MUC5AC [95] via an EGFR and COX-2-mediated pathway. Patients with hereditary non-polyposis colorectal cancer whose basic genetic defect lies in the MMR genes appear to have a reduced expression of COX-2 in their tumours when compared with those individuals with sporadic colorectal cancers [116, 117]. Defects in MMR genes have been noted to increase an individual's susceptibility to gallbladder cancer [63]. Moreover, loss of O^6-methylguanine-DNA methyltransferase, a DNA repair enzyme, as well as MMR proteins (hMLH1 and hMSH2) were associated with a poor prognosis in gallbladder cancer [64]. MSI has been noted in gallbladder cancer. Thus, one potential explanation for the loss of protective mucin MUC5AC in the later stages of carcinogenesis could be the result of defects in MMR leading to a reduced expression of COX-2 and hence MUC5AC.

how can we further improve the model to strengthen its clinical relevance?

Variations in mutation frequency in gallbladder cancer have been attributed to geographical and ethnic variability in the disease. Non-uniformity of technique between different laboratories could also be contributory [118]. However, the appreciation of tumour clonality and tumour heterogeneity in solid organ cancers [119, 120] adds a new dimension to the perceived 'variations in reported mutations'. We have no reason to not believe that gallbladder cancer, too, would exhibit clonality and tumour heterogeneity. The advances in cancer genomics (next-generation sequencing) will most likely aid in delivering critical insights into all stages of tumour progression establishing additional genetic determinants driving the process of carcinogenesis. Cutting edge technology and informatics can potentially elucidate the steps in carcinogenesis quicker, more efficiently and possibly even cost-effectively, making it possible to characterize the biology of primary as well as recurrent gallbladder cancer to an extent far surpassing the extent of our current knowledge. Using contemporary technologies, one can go both deep and wide into cancer genomics with whole-genome studies, targeted gene profiling, gene expression and epigenetic analysis.

implications of developing a successful model

patients with an inherited risk of gallbladder cancer

Gallbladder cancer is known to occur as part of hereditary cancer syndromes such as Lynch syndrome [121], neurofibromatosis 1

Table 2. Individual studies reporting novel alterations in premalignant and malignant tissues of gallbladder cancer along with their proposed significance [102–111]

Marker	Normal	Chronic cholecystitis	Adenocarcinoma	Feature	Reference
HMGA2	—	14.3%	59.3%	Directly correlated with size of the tumour (>2 cm), lymph node metastases, poorer differentiation and regional tissue invasion	[110]
CD9	—	88.6%	52.8%	Inverse correlation with size of the tumour (>2 cm), lymph node metastases, poorer differentiation and regional tissue invasion	[110]
CD146	—	5.7%	53.7%	Directly correlated with size of the tumour (>2 cm), lymph node metastases, poorer differentiation and regional tissue invasion	[107]
ERCC1	95%	—	53%	ERCC1 expression correlated with better differentiation and in subserosal (T2) tumours, ERCC1 staining was associated with a better survival	[111]
EZH2	—	0%	53.7%	EZH2 over-expression is associated with poor prognosis	[105]
PTEN	—	100%	48.2%	PTEN loss of expression is associated with poor prognosis	[105]
pERK1/2	—	11.4%	58.3%	p-ERK1/2 over-expression correlated with decreased survival	[104]
PI3-K	—	8.6%	50.9%	PI3K may contribute to gallbladder carcinogenesis	[104]
Hedgehog pathway				Hedgehog pathway is frequently expressed in gallbladder cancer and is associated with poorer survival	[103]
Shh	0%	—	81.7%		
Ptch1	0%	—	75.3%		
Gli1 protein	0%	—	70%		
RKIP	—	100%	57.7% (tumour); 31.2% (lymph node)	Loss of RKIP may contribute to tumour invasiveness and metastasis and is associated with reduced survival	[102]
RegIV	—	11.4%	53.7%	Directly correlated with lymph node metastases, poorer differentiation, and regional tissue invasion	[108]
MK1	—	14.3%	62%	Inverse correlation with lymph node metastases, poorer differentiation, and regional tissue invasion	[108]
PEG10	—	5.7%	48.1%	Directly correlated with lymph node metastases, poorer differentiation, regional tissue invasion and poorer survival	[106]
TSG101	—	5.7%	47.2%	Directly correlated with lymph node metastases, poorer differentiation, regional tissue invasion and poorer survival	[106]
VHL	—	88.6%	48.1%	Positive expression of VHL significantly associated with differentiation, tumour mass, lymph node metastasis and invasion of adenocarcinoma	[109]
HIF1α	—	14.3%	53.7%	Negative expression of HIF-1α significantly associated with differentiation, tumour mass, lymph node metastasis and invasion of adenocarcinoma	[109]

HMGA2, high mobility group protein A2; CD9, mobility-related protein 1; CD146, melanoma cell adhesion molecule; ERCC-1, excision repair cross-complementing group 1; EZH2, histone-lysine *N*-methyltransferase; PTEN, phosphatase and tensin homologue; pERK1/2, extracellular signal-regulated kinase; PI3-K, phosphatidyl inositol-3 kinase; Shh, sonic hedgehog; Ptch1, Shh receptor–Patched; Gli1 protein, Shh downstream transcription factor; RKIP, Raf-1 kinase inhibitory protein; RegIV, member of regenerating gene family; MK-1, type 1 transmembrane protein (Ep-CAM); PEG10, retrotransposon-derived protein 10; TSG101, tumour susceptibility gene 101; VHL, Von Hippel–Lindau gene; HIF1α, hypoxia-inducible factor 1 α.

[122] and Gardner's syndrome [123]. A nation-wide study from Sweden [124] concluded that the risks of familial clustering of gallbladder cancer cases were so high to suggest a contributory role, albeit modified by environmental factors. They noted that demonstration of candidate genes would help further characterize the familial risks. Developing a complete understanding of the genetic transformations involved in gallbladder carcinogenesis could aid in selecting markers for screening the disease, especially in regions of high incidence of gallbladder cancer. It can only be conjectured at this point that the determination of such markers could guide decision-making regarding prophylactic cholecystectomy in mutation carriers.

patients with surgically resectable gallbladder cancer

The development of prognostic markers could aid in deciding which patients with node-negative disease would benefit from adjuvant treatment. Besides, decisions on the choice of therapy in patients with node-positive disease could be decided based on biomarkers.

patients with metastatic gallbladder cancer

Identification of prognostic markers could aid in deciding which patients would benefit from specific palliative strategies (chemotherapy, radiotherapy and/or targeted therapies), thereby permitting the optimal use of resources and finances. Traditionally, the management of advanced gallbladder cancer involved chemotherapeutic agents like gemcitabine and 5-fluorouracil. However, recent reports on the potential role of targeted therapies [125–127] using anti-angiogenic, anti-HER-2/neu or novel MAPK/ERK kinase (MEK) inhibitors lends further support to the concept of the tumorigenesis model to permit a better approach to developing treatment strategies.

This review provides an up-to-date, evidence-based model of gallbladder carcinogenesis and its dissemination. It serves as the scaffolding for the eventual complete elucidation of the exact mechanisms involved in gallbladder carcinogenesis. We foresee the development of molecularly based individualized cancer care as a result of further elucidation of this tumorigenesis pathway using conventional as well as broad-based genomic platforms. Such an approach could not only enable, but reinforce, a cyclical process of selecting treatment for an individual patient based on the genetic expression, proteomic profiles, deregulated cellular pathways and/or somatic mutations in cancer cells of each individual patient, using this profile to accurately define the prognosis in these patients, and suggesting treatment options or clinical trials that are most likely to succeed—something that can be related with the pathological heterogeneity in clinical response frequently observed in clinics, thereby setting us on the vanguard of transforming the current cancer treatment paradigm.

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disclosure

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