Understanding the mechanisms of gallbladder lesions: A systematic review

ANDREI BOJAN[1], LILIANA GEORGETA FOIA[1], MARIA CRISTINA VLADEANU[2], IRIS BARARU BOJAN[2], CARMEN PLESOIANU[3], ALEXANDRU PLESOIANU[1] and CATALIN PRICOP[1]

[1]Department of Surgical Sciences, 'Grigore T. Popa' University of Medicine and Pharmacy; Departments of [2]Pathophysiology and Morpho-Functional Sciences and [3]Internal Medicine, Faculty of Medicine, 'Grigore T. Popa' University of Medicine and Pharmacy, 700115 Iasi, Romania

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Abstract. The gallbladder undergoes different types of pathologies, ranging from inflammatory to preneoplasia and finally to malignant lesions. Gallbladder carcinoma can be highly invasive, and it is known that chronic inflammation of the gallbladder can lead to preneoplastic abnormalities and subsequently malignant phenotypes. Gallbladder neoplasia has a low incidence but is associated with a very poor prognosis. An early diagnosis is therefore extremely important in order to improve the prognosis of patients. Immunohistochemical markers of the mucin family can distinguish between different types of gallbladder lesions. Mucins are glycoproteins that can be attached to threonine residues that are *O*-glycosylated (due to the hydroxyl group of this amino acid). Mucins are divided into two types: those that bind to the membrane, such as MUC1, and those that form gels or are secreted, such as MUC5AC. Various alterations in mucin expression have been revealed to be associated with the development of neoplasia, as they modulate cell growth, karyokinetic transformation, dedifferentiation, adhesion, invasion and immune surveillance. p53 is a tumor suppressor gene and is linked to the development of different types of neoplasia. The incidence of the p53 gene is variable in the pathophysiology of gallbladder cancer. Several studies have revealed an incidence of ~50% of the p53 gene in gallbladder tumors. Studying the immunohistochemical profile of mucins and the presence of different gene mutations in neoplastic lesions of the gallbladder and surrounding mucosa may contribute to the understanding of the pathophysiology of the disease and the mechanisms involved in tumor development, allowing the identification of patients at increased risk of developing neoplasia, thus leading to improved management.

Correspondence to: Professor Liliana Georgeta Foia, Department of Surgical Sciences, 'Grigore T. Popa' University of Medicine and Pharmacy, 16 Universității Street, 700115 Iasi, Romania
E-mail: lilifoia@yahoo.co.uk

Dr Iris Bararu Bojan, Department of Pathophysiology and Morpho-Functional Sciences, Faculty of Medicine, 'Grigore T. Popa' University of Medicine and Pharmacy, 16 Universității Street, 700115 Iasi, Romania
E-mail: iris_bararu@yahoo.com

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Contents

1. Introduction

The gallbladder may present different types of pathologies, ranging from inflammatory to preneoplasia and finally to malignant lesions. Gallbladder carcinoma is highly invasive and it is known that chronic inflammation of the gallbladder can lead to preneoplastic abnormalities and subsequently malignant phenotypes. The incidence of neoplastic gallbladder displacement is not high but is associated with a very poor prognosis. Therefore, early diagnosis is extremely important to improve the prognosis of patients. Most patients present late, after the malignant tumor has spread, when complete surgical removal of the tumor is complicated or rather impossible (1-3).

The presence of gallstones or chronic cholecystitis are the pathologies most commonly associated with gallbladder cancer, being present in 60-90% of cases. In up to a third of cases, gallbladder neoplasia may be associated with xanthogranulomatous cholecystitis (XGC). However, further studies are required to assess the link between XGC and gallbladder neoplasia.

There are different types of markers that may play a role in the pathophysiology of this type of neoplasia. Certain studies (4-6) have highlighted the link between p53 tumor suppressor gene abnormalities and the development of malignant lesions in the gallbladder, but the diversity of research focusing on this topic remains unsatisfactory. Carbohydrate

antigen 19-9 (CA 19-9) and carcinoembryonic antigen (CEA) are commonly used markers for assessing digestive karyokinetic processes. In addition, abnormalities of CEA have also been identified in gallbladder carcinoma, but there is no clear data on its involvement in benign forms of gallbladder pathology such as chronic cholecystitis and XGC (4-6).

Gallbladder neoplasia is a very aggressive form of malignancy and has an abysmal prognosis. During childhood it is silent and for most of its evolution it is asymptomatic until the disease becomes very aggressive and reaches advanced stages that cannot be cured, with palliative management as the only available strategy. It is of real concern that the overall median survival rate for patients with gallbladder cancer is 6 months, with a 5-year survival rate of 5% (7).

The high mortality rate may be a consequence of the aberrant anatomy of the gallbladder, whose wall lacks a serous layer adjacent to the liver. The connective tissues of the gallbladder and liver are contiguous, thus facilitating invasion and progression of metastases. The epidemiology of this malignancy is variable in different countries, but mortality rates have been said to closely follow incidence: Countries with the highest incidence experience the highest mortality. Worldwide, the Mapuche Indians of Chile have the highest incidence and mortality (35 per 100,000 each year), followed by the Hispanic population and by the North Ameridians. At diagnosis <10% of the lesions can be resected and 50% of patients have metastasis in the lymph nodes (8-10).

Despite the increasing trends of gallbladder neoplasia in certain countries and its poor prognosis, there are only a few studies in the literature searching for the most appropriate markers more commonly associated with this type of malignancy, which could guide to an earlier diagnosis. Therefore, the present study was undertaken, in the form of a systematic review, in an attempt to identify the markers that are most commonly associated with a form of gallbladder dysplasia or karyokinesis.

Science Direct, Scopus, PubMed and Google Scholar were used to search for original articles and review articles published in English with the following key words: 'biliary dysplasia', 'markers' and 'cholecystic neoplasia'. Scientific databases were searched from January 2000 to January 2020. Only full-text articles were selected. Duplicate publications, irrelevant topics and book chapters were excluded.

2. Immunohistochemical markers

Immunohistochemical markers of the mucin family may determine different types of gallbladder lesions.

Mucins refer to glycoproteins containing threonine residues that are *O*-glycosylated. These molecules are of two types: MUC1 and MUC5AC. MUC1 are membrane-binding molecules, while MUC5AC are secreted or gel-forming molecules. The varied expression of these types of mucins may be associated with tumor progression, as mucins have been revealed to modulate cell growth and neoplastic transformation, influencing processes such as dedifferentiation, adhesion and immune surveillance (11).

MUC1 is located in the transmembrane region and is observed on the apical surfaces of most epithelial cells and therefore may be found in tissues located in the breast, digestive structures, respiratory system or genitourinary tract. The gene for the transmembrane mucin MUC1 is located on chromosome 1q21-24 (10). The secreted form of mucin, which is represented by MUC5AC, with its gene on chromosome 11p15.5, is usually identified in the stomach and respiratory tract (12). This type of mucin is not observed in healthy digestive tissues but is frequently detected in colorectal neoplasia (13).

Regarding the involvement of these types of mucins, some research has revealed that gallbladder inflammation is associated with an increased level of MUC5AC expression (3,14).

In regard to the levels of these mucin types, MUC1 and MUC5AC have been shown to influence the development of malignant tumors, being able to interact with the biological behavior and progression of gallbladder adenocarcinoma (15). MUC1 expression may be considered as a marker of neoplastic transformation and extension affecting the gallbladder epithelium. MUC1 has also been revealed to be related to tissue invasion, lymphatic metastasis and appears to induce a non-papillary type of carcinoma (16). Even more so in digestive adenomas and dysplasia, MUC5AC levels tend to decrease, while MUC1 has an increasing trend.

MUC1 and MUC5AC expression is assessed by immunohistochemistry and various studies have indicated that MUC1 expression increased as the severity of gallbladder lesions progressed from hyperplasia to carcinoma *in situ*, while MUC5AC expression decreased as the severity of lesions increased (16-18). MUC1 expression is clearly higher in gallbladder adenocarcinoma than in chronic cholecystitis, whereas MUC5AC expression is lower in neoplasia than in chronic inflammation (16).

3. Genetic variation in the pathogeny of gall bladder neoplasia

Epithelial dysplasia and metaplasia are changes described in both inflammatory and malignant lesions of the gallbladder. The incidence of dysplasia has been revealed to be higher in gallbladder cancer (studies revealed an incidence of 74% in dysplastic lesions of gallbladder malignancy and only 28% in benign gallbladder lesions). Antral metaplasia has been found in more than half of the cases of chronic cholecystitis, including XGC. However, Agrawal *et al* reported that the prevalence of metaplastic lesions is similar in gallbladder cancer and chronic inflammatory lesions (6).

In order to analyze a tumorigenesis pattern as in colorectal cancer, it would first be appropriate to assess whether gallbladder cancer evolves from benign lesions to malignant transformation in such a staged manner that could be similar to that of colorectal cancer, i.e., a transformation from adenoma to carcinoma. Research conducted by Laitio provided a milestone in understanding the pathogenesis of gallbladder cancer (19). Other authors, such as Albores-Saavedra *et al* and Roa *et al* have supported the theory of progressive transformation of lesions and eventually succeeded in demonstrating the presence of metaplasia, dysplasia and carcinoma *in situ* near invasive carcinoma, thus highlighting the importance of the metaplasia-dysplasia-carcinoma cascade (20,21).

Studies by Nakajo *et al* suggested that both metaplastic and non-metaplastic adenomas can progress to adenocarcinomas. Therefore, it has been endorsed that there are two

distinct pathways in the induction of gallbladder cancer: The dysplasia-carcinoma sequence (common in patients with gallstones) and the adenoma-carcinoma progression (2,22). Of these two hypotheses, the most plausible type of progression remains the transformation from dysplasia to carcinoma, as adenomas have only a minor incidence and may be present in close proximity to both early and advanced forms of gallbladder cancer. Furthermore, there is some evidence suggesting that different mechanisms are responsible for the development of adenoma compared with adenocarcinoma formation (22).

The p53 tumour suppressor gene may lead to the development of different types of malignant tumors. The presence of the p53 gene in gallbladder cancer is variable and may be as high as 50%, as certain studies have demonstrated (4,5). Previous research has revealed an even higher incidence of p53 in gallbladder neoplasms, reaching ~70% (23). Only a few studies have evaluated the incidence of p53 in precursor lesions of gallbladder karyokinesis. p53 overexpression in dysplasia may be associated with both the neoplastic phenotype and the chronic inflammatory pattern. In gallbladder cancer-associated dysplasia, p53 overexpression was identified in 17% of low-grade dysplasia lesions and in 40% of high-grade dysplasia lesions. Dysplasia observed in chronic cholecystitis revealed a 14% incidence of p53 expression. Normal gallbladder tissue and metaplastic or non-metaplastic lesions in non-neoplastic and neoplastic gallbladder pathologies, including XGC, were negative for p53 (24,25). There is one study (26) that reported a significant correlation between p53 overexpression and the presence of gallstones, stage of tumor lesions, tumor aggressiveness and liver invasion, while other researchers have not proven a significant correlation between p53 overexpression in gallbladder neoplasia and clinical parameters such as age, sex, histological type and extent of tumor invasion and neoplastic stage (25-27).

Not only are abnormalities in tumor suppressor genes responsible for the induction of gallbladder neoplasia, but also other genetic changes such as the presence of oncogenes, abnormalities in DNA repair genes, the presence of microsatellite instability or aberrant methylation of gene areas (27).

KRAS is an oncogene that is implicated in numerous types of malignancies since it plays a role in signal transduction mechanisms that may be disrupted in neoplastic progression. In gallbladder cancer, mutations in the KRAS gene mainly affect codons 12, 13 and 61. These mutations trigger altered growth signals that are essential for malignant transformation (27).

Another genetic alteration that can be identified in the malignant genome refers to loss of heterozygosity (LOH). This abnormality may occur in the case of heterozygous deletion of alleles or in the case of duplication of a chromosomal region that induces subsequent deletion of other alleles. Studies have revealed abnormalities such as LOH in the 3p, 8p, 9p and 22q regions (9,28-30).

4. Other markers related to gall bladder tumors

To date, there are no specific tumor markers for gallbladder cancer. CEA and CA 19-9 can be detected in high concentrations in advanced neoplastic stages, but these abnormalities have low specificity. Cancer antigen 125 (CA 125) is a glycoprotein that is commonly used as a tumor marker for ovarian malignancies, while certain studies have suggested that its levels may be altered in gallbladder tumors. Research conducted by Kumar *et al* proposed a triple assessment of the tumor markers CEA, CA 125 and CA 19-9 to predict the risk of gallbladder carcinoma (30). The results revealed that these markers have high specificity in advanced-stage neoplasia, but low efficiency in predicting metastatic gallbladder cancer (30-35).

5. Conclusions

The analysis of different markers, such as the immunohistochemical profile of mucins, identification of genetic changes and the anomalies of different serological markers, may lead to a deeper understanding of the pathophysiology of gall bladder cancer allowing further identification of patients at increased risk of developing neoplasia, which could allow improved management of the disease.

While the exact mechanisms involved in gallbladder karyokinesis remain unresolved, the present review highlighted some of the most important pathways associated with gallbladder pathologies. Conventional and extended proteomic and genomic platforms could lead to an individualized molecular-based approach to neoplasia in the future.

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All data generated or analyzed during this study are included in this published article.

Authors' contributions

AB, LGF and IBB searched the literature and wrote the first draft of the manuscript. CPl, AP and MCV collected and interpreted the data regarding the genetic variations in gallbladder lesions. LGF and CPr designed the study and supervised the whole review. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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