



CYCLOOXYGENASE-2, P53 AND GLUCOSE TRANSPORTER-1 AS PREDICTORS OF MALIGNANCY IN THE DEVELOPMENT OF GALLBLADDER CARCINOMAS

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ABSTRACT

Gallbladder carcinoma is the fifth most common malignancy of the gastrointestinal tract. The absolute characteristics of the disease are the high mortality rate due to the late discovery of a tumor and the low therapeutic possibilities except by surgical intervention. In oncology we can predict the outcome of the disease with a combination of classical standard clinico/pathological parameters (stage of the tumors, differentiation) and the intrinsic genetic and biochemical properties of the tumor. Such intrinsic properties of the tumors that are connected with the outcome of the disease are the denominators (markers). The author searched extensively for the expression and influence of 3 markers included in chronic inflammation and early carcinogenesis, cell cycle regulation and tissue hypoxia: cyclooxygenase-2 (COX-2), p53 gene and glucose transporter-1 protein (GLUT-1). The author discusses their possible role in the development as well as fighting this disease, if specific medications targeting them were available.

KEY WORDS: gallbladder carcinoma, cyclooxygenase-2, p53, glucose transporter-1

INTRODUCTION

Mysterious and hidden in the abdominal cavity, gallbladder malignancies have continued to be a puzzle and an under-investigated disease. However, gallbladder carcinomas are the 5th most common malignancy of the gastrointestinal tract. Histologically, in 95 % of cases there is an adenocarcinoma. It is commonly detected late, after a quiet period, many times incidentally, so it is no surprise that this malignancy has a poor overall survival of 5-13 % for 5 years (1). Because next to surgical resection there are no relevant guides for additional therapy, the situation calls for investigations of early markers (and/or triggers) of this disease and with them - new therapeutic possibilities. Early observations directed the attention towards an association with cholelithiasis (2), in the Far East with an anomalous pancreatico-biliary ductal junction (APBDJ) (3), together with a common denominator: long-term inflammation as a cause. Histological examinations revealed that epithelial changes and premalignant lesions were found adjacent to the tumor tissue (4). Nowadays, in contemporary pathology, 3 putative pathways of gallbladder carcinogenesis are accepted: dysplasia, adenoma and APBDJ (4). Next to these putative pathways, diseases which are probably connected to gallbladder carcinomas are: porcelain gallbladder, ulcerative colitis, familial adenomatous polyposis, Gardner's and Peutz-Jeghers syndrome. In our part of the world, the first pathway of gallbladder tumorigenesis, dysplasia, is the most widespread. The most important basis for putting dysplasia in the position of premalignancy is that the detection rate of dysplasia adjacent to a carcinoma is 45-88%, since solitary dysplasia is found in 0.4-33% of cases (4). Many times in dysplastic areas metaplasia is detected, with typical intestinal and pyloric glands. Coming from the assumption that the period required to transform into an advanced carcinoma is 15 years (5), nowadays we know how important it is to find factors that trigger the carcinogenic process. These potential early markers in the development of gallbladder cancer are maybe at the same time also possible targets for therapeutic intervention. Roa has already postulated that in certain countries (such as Chile, where there is a difference in the length of time of the expression of gallbladder carcinomas between two ethnic groups, where the period of time from dysplasia to carcinoma in Mapuche Indians is half as much as the other group) an effort should be made to determine the environmental and nutritional risk factors 20 years before the appear-

ance of advanced carcinomas, and that in these factors there might be a clue for difference in the different speed of the carcinoma development. Nevertheless, in contemporary pathology the attention has been directed mostly towards the intrinsic genetic and biochemical properties of the tissue and so our research was focused on COX-2, the p53 gene and the glucose transporter-1.

COX-2

COX is a rate-limiting step in PGH₂ synthesis. It is presented in 2 isoforms, from which one is constitutional: COX-1 is normally present and obligatory for maintenance of gastrointestinal mucosis, platelet function and renal function. However, COX-2 is induced by inflammatory and carcinogenic stimuli. Once COX-2 is presented in the tissue, it has the power to promote tumorigenesis by enhancing angiogenesis, stimulation of Bcl-2 transcription, suppression of the cell-mediated anti-tumor immune response, induction of matrix metalloproteinase, induction of cell proliferation and invasion and through the activation of several classes of chemical carcinogens (6-8). COX-2 overexpression was detected in several malignant and premalignant conditions (9), also in gallbladder carcinomas (10-12). Asano (10) observed in advanced gallbladder carcinomas an enhanced expression of COX-2 in the adjacent stroma rather than in the cancerous epithelia. The stroma was a potent source of PG synthesis. In the study by Kawamoto et al (13) the expression of COX-2 in the subserosal layer of the gallbladder tumor stroma correlated with the aggressiveness of the disease. In our study (11) we included surgical specimens (tissue samples) of carcinomas, high grade dysplasias, low grade dysplasias, hyperplasias and normal gallbladders. A typical cytoplasmatic pattern of COX-2 staining was shown either in tumor cells, sometimes in stromal fibroblasts or smooth muscle cells, endothelial or epithelial cells. For evaluating the degree of COX-2 expression we used an immunoreactive score, the product of the intensity of staining and the quantity of positive cells. The highest IRS was found in the high-grade dysplasia and not in the carcinoma. The highest COX-2 expression that appeared in the premalignant condition led us to the conclusion that COX-2 overexpression is an early occurrence in gallbladder carcinogenesis (11). Our observation was in accordance with tumor behaviour in gastric cancer (14), where enhanced COX-2 expression was also an early occurrence in tumorigenesis. Our study is also supported by the findings of Fumino et al (12) who detected enhanced an expression of COX-2

in the mucosal hyperplasia of the gallbladder in patients with an anomalous arrangement of the pancreaticobiliary duct, suggesting that COX-2 might play a regulatory role in the proliferation of gallbladder epithelia.

P53

The p53 gene is known as the guardian of the genome. Upon receiving a signal of DNA damage, the p53 amplifies and becomes active in the cell to induce transcription, prolong the cell cycle and repair the DNA or induce apoptosis rather than repair, so that the damaged cells are removed from the tissue (15). Wild-type p53 is thus an oncosuppressor gene, known for its instability and difficulty in immunohistochemical showing in the cell nucleus. However, a mutant p53 is a stable protein with a long half-life, possible to reveal its presence in the cells. An increased p53 level in the cell can be used as an indicator of p53 mutation. P53 gene mutation or deletion is observed in 50% of cancers (16). P53 expression was found to be negative in the normal gallbladder epithelium, hyperplasias and low-grade dysplasias, but positive in an important percentage of high-grade dysplasia of gallbladder epithelia (31,2%) and in 48,1% of gallbladder carcinomas (11). Our results were in accordance with previous studies where the incidence of p53 accumulation in gallbladder carcinoma was 36-68% (17-19). There are links between COX-2 and p53 expression. COX-2 might be suppressed by wild-type p53 (20), COX-2 in turn inhibits the wild-type p53 dependant transcription (21). We were the first to analyse the relationship between COX-2 and p53 in various histological stages of gallbladder epithelial abnormalities (11). Putting on one side all p53 negative cases and on the other side p53 positive cases, we found that among the p53 positive cases there were only 2 COX-2 negative cases, all others were positive too, and among the p53 negative cases there were 60% COX-2 negative. This was statistically significant. The earlier appearance of COX-2 expression has led us to presume that COX-2 overexpression might be the first occurrence that in turn inhibits wild-type dependant transcription. We conclude that COX-2 overexpression might be related to p53 dysfunction (11). This has a great therapeutic impact, because targeting COX-2 might be beneficial, affecting 2 regulatory molecules: COX-2 and p53. The use of non-steroid anti-inflammatory drugs and COX-2 selective inhibitors in human cancers were found promising in the chemoprevention of carcinomas. The use of selective COX-2 inhibitors (celecoxib) decreased the number and size of colon

polypees in familial adenomatous polyposis within 6 months of treatment (22). The following studies (23, 24) showed a significantly higher incidence of cardiovascular occurrences in these patients, so the chemoprevention of colorectal carcinomas with them is not justified. The complex role of COX-2 in the physiological and pathophysiological processes in humans is highly suggestive and merits further investigations.

GLUT-1

Accelerated glycolysis is one of the biochemical characteristics of cancer cells (25). GLUT-1 is a transmembrane glucose transport protein that allows the facilitated transport of glucose into cells. Next to its normal expression in tissues which depend mainly on glucose metabolism, such as membranes of erythrocytes, the endothelium of brain capillaries, perineurium, renal tubules, it is also found also as an adaptation mechanism of tumor cells to ensure glucose transport (25, 26) and it is recognized as a successful model that leads to invasion and metastasis (27, 28). It has been shown that it is occasionally present in reactive benign epithelia in small quantities, but in increased expression in various malignoma: head and neck squamous cell carcinomas, gastric carcinomas, colorectal, ovarian, endometrial, also in gallbladder carcinomas (29-31). Nowadays we know that hypoxic stimuli accelerate GLUT-1 overexpression and increased expression of GLUT-1 is a marker of hypoxia (32). Since Kim et al. (30) showed accelerated GLUT-1 expression in gallbladder carcinomas, Legan et al. (31) proposed that the gradual increase in GLUT-1 expression appears from a low grade dysplasia towards a carcinoma. Since strong GLUT-1 expression appears only in carcinoma cases, we believe that our results have compelling diagnostic importance. This means that the specificity of strong GLUT-1 expression for detecting gallbladder carcinomas is 100% (31). In contemporary pathology sometimes there is a puzzle to decide between high-grade dysplasia and a carcinoma in situ. In such cases when the Asian school always tends towards a carcinoma, we now have a complementary marker for facilitating a decision. However, the diagnostic puzzle remains in the GLUT-1 negativity, which was the case in 48,2% of gallbladder carcinomas (31). Patients, whose tumors strongly expressed GLUT-1, had significantly shorter survival times than patients with absent, weak or moderate GLUT-1 expression (33). We confirmed that GLUT-1 is not only a marker of hypoxia, but also an obvious denominator of the

poor prognosis in gallbladder carcinoma patients. Pharmacological inhibition of glucose metabolism has been shown to exhibit promising anticancer activity and the GLUT-1 receptor could be a pharma-

ceutical target for potential anticancer therapy. However, GLUT-1 has a crucial role in supplying the brain with glucose, so the main effort will be to find a molecule that cannot pass the haemato-encephal barrier.

CONCLUSION

A gradual increase in the accumulation of GLUT-1 (or p53 and/or COX-2) was observed from low-grade dysplasias to carcinomas. Since COX-2 and p53 are involved in the carcinogenesis of the gallbladder itself, GLUT-1 is a consequent occurrence of malignant or premalignant alterations. When once present it becomes more than just a diagnostic marker in certain dubious cases but also a prognostic marker unmasking hypoxic conditions and through them the unfavourable biological behaviour of the tumor. Early markers and denominators are important because they give the opportunity to detect early occurrences in the development of gallbladder carcinoma. Targeting them might be beneficial in patients with gallbladder carcinomas and it is the subject of present and future investigations.

List of Abbreviations

COX-2 - cyclooxygenase-2
GLUT-1 - glucose transporter-1 protein
APBDJ - anomalous pancreatico-biliary ductal junction
IRS - immunoreactive score

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