Review Article

Molecular Biology of Gallbladder Cancer: Potential Clinical Implications

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Abstract

Gallbladder cancer (GBC) is a common malignancy of the biliary tract and involves the changes in multiple oncogenes and multiple genetic genes. Since over the past decade there has been an advance in the knowledge of the genetic basis of cancer, mainly as a result of the rapid progression of molecular technology; however, conventional therapeutic approaches have not had much impact on the course of this aggressive neoplasm. Knowledge of the molecular biology of GBC is rapidly growing. Genetic alterations in GBC include adenosine triphosphate-binding cassette transporter ABCG8, membrane-bound enzyme ADAM-17 of multi-functional gene family, and other genes including p53, COX2, XPC, and RASSF1A. The advances in molecular biology have potential implications for the detection of this disease, using Synuclein-gamma, Syndecan-1, glycoprotein 72 (TAG-72), tumor endothelial marker 8 protein (TEM8) and TNF-alpha. The use of these molecular diagnostic methods is of clinical importance for the gene replacement therapy, genetic prodrug activation therapy, and antisense immunology technology for the treatment of malignancy. The author reviewed recent publications on PubMed, and summarized molecular biology of GBC, with an emphasis on features of potential clinical implications for diagnosis and management.

Keywords: Adenosine triphosphate-binding cassette transporter, Gallbladder cancer, Syndecan, Synuclein-gamma, TEM8, TNF, XPC

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Introduction

Gallbladder cancer (GBC) shows a marked geographical variation in its incidence, with the highest figures being seen in India and Chile, and relatively low levels in many Western countries. Risk factors for its development including the presence of gallstones, infection, and anomalous pancreatobiliary ductal junction are well documented, and a number of genetic alterations have also been identified in the pre-invasive and invasive stages of GBC. Some of these genetic changes are associated with particular risk factors, and some changes are associated with differences in prognosis. GBC involves the changes in multiple oncogenes and genetic genes.[1] The article summarized more recent publications on PubMed, specially in the molecular biology of GBC, with an emphasis on clinical implications.

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Difference between early and advanced stages

It has been shown that genetic expressions vary in early and advanced GBC.[2] GBC usually arises against the background of gallstone disease, which may be causatively related to supersaturation of cholesterol in bile. The excretion of cholesterol from the liver is regulated by adenosine triphosphate-binding cassette transporter ABCG8. A common genetic polymorphism D19H of ABCG8 and Apolipoprotein B (APOB) associated with gallstone disease may be related to the genetic predisposition of GBC. The ABCG8 DH genotype frequency and APOB was significantly higher in GBC patients. The risk was more pronounced in GBC patients with gallstones, and in patients with an early onset of the disease. Therefore, the DH genotype and the H allele of the ABCG8 D19H polymorphism are associated with GBC susceptibility. The GBC patients with gallstone disease harboring the ABCG8 variant allele and APOB[3] are at a higher risk.[4]

ADAM-17, known as tumor necrosis factor-alpha converting enzyme, is involved in the progression of GBC. It has been shown that expression of ADAM-17 is significantly increased in tumors with high histological grade and pT stage compared with low histological grade and pT stage tumors. Patients with high expression of ADAM-17 had a significantly shorter overall survival compared with those with low expression.[5,6] Moreover, annexinA3 is found to play an important role in the initiation and progression of GBC.[7] These are of importance for the potential molecular targets for diagnosis and treatment of GBC.

Bas excision repair

Base excision repair (BER) corrects DNA damage caused by oxidative stress and chronic inflammation, putative risk factors for cancer. To understand the relationship between genetic variation in BER genes and risk of biliary tract cancer and biliary stones, it was examined nonsynonymous polymorphisms in three key BER genes-X-ray repair cross-complementing group 1 (XRCC1) (R194W, rs1799782; R280H, rs25489 and R399Q, rs25487), apurinic/apyrimidinic endonuclease (APEX1) (D148E, rs3136820), and 8-oxoguanine DNA glycosylase (OGG1) (S326C, rs1052133). Compared with subjects carrying the XRCC1 194RR genotype, those with the WW genotype had a 1.9-fold risk of bile duct cancer and compared with subjects carrying the XRCC1 280RR genotype, those with the XRCC1 280H allele had a 50% reduced risk of bile duct cancer. It was also found an inverse association between the APEX1 148E allele and gallbladder stones, but no association for the OGG1 polymorphism.[8]

Deregulation of apoptosis is involved in the mechanisms of cancer development. Bax-interacting factor-1 (Bif-1) interacts with both Bax and Bak that are crucial for the intrinsic apoptosis signaling. Functionally, loss of Bif-1 expression has been proven to enhance tumorigenesis, possibly by inhibiting apoptosis mediated by Bif-1.[9]

Cadherin

E-cadherin/beta-catenin

Changes of the E-cadherin/beta-catenin complex during cell–cell interactions result in loss of cell adhesion, and may account for the ability of cancer cells to metastasize. The beta-catenin membranous expression decreased between cholecystitis and malignant tissue, as well as between normal epithelium and carcinoma. The E-cadherin membranous expression was reduced in normal gallbladder epithelia compared with carcinoma. Cytoplasmatic E-cadherin was significantly different from normal gallbladders to carcinomas and between normal tissue and inflammation. Therefore, significant differences of E-cadherin and beta-catenin were detected between normal, inflamed, and cancerous tissues. These changes of the protein expressions and the associated loss of adhesive mechanisms might lead to a cancerous pathway in GBC.[10] Loss of E-cadherin expression was high in GBC, while majority of the chronic cholecystitis (CC) and xanthogranulomatous cholecystitis (XGC) cases retained positive E-cadherin expression.[11] Moreover, the expression of E-cadherin or beta-catenin frequently diminishes as the tumor progresses, and abnormalities of E-cadherin and beta-catenin expression were associated with decreased apoptosis in GBCs. E-cadherin expression might be a useful prognostic marker in the tumor.[12]

T-cahderin

T-cadherin is believed to act against carcinogenesis in various tissues; however, the tumor-suppressor mechanism of T-cadherin remains largely unclear. Restoration of T-cadherin decreased the expression of Akt3 and phosphorylated Akt molecules. T-cadherin may inhibit tumor progression through multiple pathways.[13] Zeb1 represses T-cadherin expression and increases the invasive activity of GBC.[14]

Cancer stem cells

CSCs/tumor-initiating cells have been defined as a subset of tumor cells responsible for initiating and sustaining tumor development. Emerging evidence strongly supports the existence of CSCs in various solid tumors, but they have not yet been identified in human GBC. However, human GBC cell line GBC-SD cells were cultured in a serum-free culture medium with different concentrations of the chemotherapeutic drug cisplatin for generating sphere clones. It demonstrated that sphere clones of GBC with stem cell-like characteristics can be obtained using suspension cultures of GBC-SD cells in serum-free culture medium containing cisplatin.[15] Alternatively, CSCs in primary GBC and in the cell line GBC-SD using the cell surface markers CD44 and CD133 were identified. These cells showed stem cell properties, including self-renewal, differentiation potential, and high tumorigenicity. *In vitro* culture experiments revealed that CD44+CD133+ GBC cells possessed a higher spheroid-colony forming ability in serum-free media than other subpopulations. These suggest that the CD44+CD133+ population exhibited CSC-like characteristics, and may provide a novel approach to the diagnosis and treatment of GBC.[16]

Cluster differentiation and natural killer cells

The patient with malignant tumor always shows immunologic function drawback and ingravescent with tumor development, especially in the aspect of cell-mediated immunity. One study was undertaken to define the relationship between the immune function of local cells and cancer development by investigating the

distribution of natural killer (NK) cells and T-lymphocyte subsets in peripheral blood, the cancer tissue and the tissue surrounding gallbladder carcinoma. This study indicated that the numbers of CD4+ and CD8+ T-cells and NK cells in GBC tissues were significantly higher than those in the surrounding tissue and gallbladder with gallstone. However, the ratio of CD4+/CD8+ was lower in the cancer tissue than that in the surrounding tissue and tissue from gallbladders with gallstones. The distribution of CD4+ and CD8+ T-cells and NK cells in mucous membrane of cholecystitis gallbladder and that in the tissue surrounding GBC were significantly different. Disproportionate and imbalanced distribution of NK cells and subsets of T-lymphocytes occurs in the mucous membrane proper of GBC and surrounding tissue. Although GBC tissue has higher expressions of CD4+, CD8+ and NK cells, the immune function is low or in an inhibited state. In GBC immunization therapy, local cellular immunological function should be enhanced and the protective barrier improved.[17]

CD24, a small cell surface protein, has emerged as a novel oncogene and prognostic factor for poor outcomes in many human cancers. CD24 is an important marker of malignancy and poor prognosis in gallbladder carcinoma. Its detection combined with cancerous staging may increase the ability of investigators to predict the prognosis of patients with gallbladder carcinoma. Furthermore, the CD24 antigen represents an attractive target for specific therapies with monoclonal antibodies in patients with CD24-overexpressing gallbladder carcinoma, so the detection of CD24 may help clinicians select patients likely to benefit from novel molecular therapies.[18]

CD133+ tumor cells are shown to be responsible for the initiation, propagation, and recurrence of tumors. Purified CD133+ gallbladder carcinoma cells are highly resistant to conventional chemotherapy. However, As2O3 effectively induces CD133+ gallbladder carcinoma cells apoptosis. Furthermore, the ectopic expression of CD133 attenuated the apoptotic effect of As2O3on cells through activation of AKT signaling pathways.[19]

CD147

Pathologic findings demonstrated that the intensity of CD147 and MMP-2 staining in cancerous tissues was associated significantly with histological types, distant metastasis, and Nevin stages of gallbladder carcinomas. Using a proportional hazard model, the survival rate of the patients with CD147+/MMP-2+ expression was the lowest, and including information on CD147 and MMP-2 staining patterns within cancerous tissues along with clinical cancer staging may improve the accuracy of predicting patients' prognosis. The detection of these two markers combined with cancerous staging may increase the ability of investigators to predict the prognosis of patients with gallbladder carcinomas.[20]

The caudal-type homeodomain transcriptional factor *CDX2*, a member of the caudal-related homeobox gene family, plays a crucial role in the regulation of cell proliferation and differentiation in the gut. CDX2 expression was an independent prognostic predictor in gallbladder adenocarcinoma. CDX2 and Hep might function as important biological markers in the development and prognosis of gallbladder adenocarcinoma.[21-23]

c-erB-2

The inactivation of the tumor suppressor gene and activation of the proto-oncogene are the key steps in the development of the human cancer. The p53 and c-erbB-2 are the best examples. p53 and c-erbB-2 may have independent role in carcinogenesis of gall bladder cancer. c-erbB-2 over expression in adenoma and younger age group indicates its role as an early event in carcinogenesis of gallbladder.[24]

c-FLIP

Cellular Fas-associated death domain-like interleukin-1 converting enzyme inhibitory protein (c-FLIP), an antiapoptotic protein, was over-expressed in the most of gallbladder carcinoma tissues. Thus, a potent strategy for the treatment of gallbladder carcinoma by targeting the c-FLIP was presented.[25]

Chloride intracellular channel 1

Advanced GBC has an extremely poor prognosis because of metastasis. Identification of metastasis-related biomarkers is essential to improve patient survival. The over expression of Chloride intracellular channel 1 (CLIC1) promoted cell motility and invasion of GBC-SD18L *in vitro*, while RNA interference of CLIC1 remarkably decreased cell motility and invasive potency of GBC-SD18H *in vitro*, indicating that CLIC1 might play an important role in metastasis of gallbladder carcinoma.[26]

Differences between gallbladder and bile duct cancers

Biliary tract cancers carry dismal prognoses. It is commonly understood that chromosomal aberrations in cancer cells have prognostic and therapeutic implications. The bacterial artificial chromosome (BAC) array comparative genomic hybridization (CGH) can facilitate detail analysis with high resolution and sensitivity. Both GBC and BDC cell lines have DNA copy number abnormalities of gains and/or losses on every chromosome, which is possible to determine the genetic differences between gallbladder and bile duct cancer cell lines.[27]

Epidermal growth factor and receptor

EGF and transforming growth factor beta1 (TGFbeta1) play important roles in tumor biology. Single nucleotide polymorphisms in EGF and TGFB1 genes alter the expression of these growth factors and influence the tumorigenesis process.[28] Increased EGF receptor expression has been noted in various cancers, and has become a useful target for therapeutic interventions. In GBC high expression of epidermal growth factor receptor (EGFR) is an independent predictor of survival.[29,30] EGFR pathway is suitable therapeutic targets for biliary tract carcinomas. The combination of gemcitabine with drugs targeting these pathways gives encouraging results and further clinical studies could be warranted.[31] Because EGF and HER-2/neu antagonists have been successfully used in adenocarcinomas from other sites, their use in cholangiocarcinoma can be potentially beneficial. Over expression of EGF correlated significantly with copy number of EGF. HER2/neu expression is uncommon in these tumors.[32]

A recent study showed that tumor samples harboring EGFR mutation had phosphorylation of one or both downstream transducers analyzed. This suggests that patients with cholangio-carcinoma or gallbladder carcinoma exhibit somatic mutations of EGFR in the tyrosine kinase domain that can elicit cell signals sustaining survival and proliferation. These tumors might be further evaluated for their susceptibility to small-molecule inhibitor treatment.[33]

Inflammation genes

Long-standing gallstones are generally present in 65-80% patients of GBC. It has also been suggested that inflammation caused by gallstones may be involved in the development of GBC. Interleukin-1 receptor antagonist (IL-1RN) and interleukin-1 beta (IL-1B) are proinflammatory cytokine genes at the interleukin-1 locus, and polymorphisms of these genes have been associated with various inflammatory diseases. Variants in genes that influence inflammatory responses may predispose to gallstones and biliary tract cancer.[34]

mda-7/IL-24 has tumor-suppressor activity in a broad spectrum of human cancer cells. It was used a human gallbladder carcinoma cell line (GBC-SD) to explore the effect of adenovirus-mediated IL-24 (Ad-IL24) gene therapy on GBC-SD cells. It was show that adenovirus (AdV)-mediated IL-24 overexpression exerted potent antitumor activity via stimulating mitochondrial apoptotic pathway in GBC-SD, and that mda-7/IL-24 has the potential to serve as a tool for targeted gene therapy in the treatment of GBC.[35] Moreover, the haplotype 1/C of IL-1RN and IL-1B was found to confer a significantly enhanced risk of GBC in cancer patients with gallstones, while higher risk resulting from 2/C haplotype was of borderline significance. Individuals with 1/C and 2/C haplotypes of IL-1RN VNTR and -511IL-1B C → T polymorphisms were more susceptible to develop GBC with gallstones than in healthy controls.[36]

Premalignant lesions

The sequence of molecular changes leading to neoplastic transformation in the gallbladder remains elusive. A study aiming to characterize the spectrum of nuclear p16 protein product immunohistochemical expression in tissue taken from resected gallbladders, comprising histologically normal gallbladder epithelia, dyplastic epithelia, reactive atypia, and gallbladder adenocarcinoma indicated that nuclear p16 expression is absent in normal gallbladder epithelium, and is a frequent event in high-grade dysplasia of the gallbladder and gallbladder adenocarcinoma.[37]

The relationship between cell cycle regulatory proteins and clinicopathologic features was immunohistochemically investigated in order to identify the biomarkers related to the outcome of patients with biliary tract cancer (BTC). Absence of p21 expression independently predicted poor outcome in all cases. Determination of p21 expression in surgically resected specimens may provide prognostic information in addition to conventional pathologic findings for patients with BTC, especially those who have biologically less aggressive phenotypes.[38] The expression of p21 between advanced stages was 90% and early stages was 54%.

The p27 expression was markedly decreased in GBC cases, and there were no significant correlation between p27KIP1 expression and all clinicopathological parameters including gender, grades, stages, and invasion, whereas the expression of p21 was 75% and there was a significant correlation between p21 and the clinicopathological parameters including gender, stages, and invasion.[39] Aberrations of p27(Kip1)-interacting cell cycle regulators are common in gallbladder carcinomas. Skp2 overexpression is highly representative of biological aggressiveness, suggesting that it is a promising novel target for therapeutic intervention in aggressive cases.[40]

Understanding the molecular events in gallbladder carcinogenesis may provide a novel targeted therapeutic approach. Alterations in the tumor suppressor gene, p53, are commonly observed in most human cancers.[41] Mutations in p53 gene are found in a majority of human malignancies and usually occur in the exons 5, 6, 7, and 8. Mutated p53 protein is more stable and gets accumulated in the cells that induce the host to develop anti-p53 antibodies in sera of cancer patients. Patients with GBC have antibodies to p53 protein. The commonest

identifiable alteration in the p53 gene is a frameshift mutation at codon 271.[42]

The microsatelital instability has been found in a small subset of preneoplastic and neoplastic lesions. The existence of methylation in the promoter gene areas has been related to the cellular proliferation, invasion and metastasis, and also in cases of chronic cholecystitis, therefore, this epigenetic phenomenon represents a crucial early event in GB carcinogenesis.[43] The p53 gene is the most frequently mutated tumor suppressor in human cancers, and the aberrant p53 expression may play a role in the occurrence of GBC.[44-46]

Mucin core proteins are known to be present in various organs, and are specifically expressed with carcinogenesis and closely associated with the prognoses of various malignant tumors. Mucin expression was independent of various tumor growth factors and clearly reflected the prognosis of GBC. Because the relative malignancy of GBC could be evaluated by examining the level of glycoprotein expression in tumor tissue, mucin could be a more important marker than p53 for predicting prognosis in gallbladder carcinoma using surgically resected tissue specimens.[47]

Molecular marker

Molecular markers for cancers are not only useful for cancer detection and prognostic prediction, but may also serve as potential therapeutic targets. The molecular markers for prognostic prediction in GBC, using by the immunohistochemical expression, mainly include 15 proteins, namely p53, p27, p16, RB, Smad4, PTEN, FHIT, GSTP1, MGMT, E-cadherin, nm23, CD44, TIMP3, S100A4, and promyelocytic leukemia (PML). In particular, PML and p53 showed considerable potential as independent prognostic markers. Patients with normal PML and p53 expression displayed favorable outcomes, compared with those showing abnormal expression of either or both proteins. PML and p53 are potential candidates for development as clinically applicable molecular prognostic markers of GBC, and may be effective therapeutic targets for the disease in the future.[48] These proteins may be useful as markers to identify premalignant lesions that are likely to progress into malignant adenocarcinoma, especially p53, Rad50, and cyclin-E proteins.[49]

GBCs are shown to frequently exhibit TP53 as well as K-ras gene mutations,[50] these findings indicate that TP53 abnormalities are early and frequent events in the pathogenesis of GBC.[51]

Conclusion

Many other molecular markers, proteins and factors, including PIK3CA,[52], methylation,[53,54] protein gene product 9.5,[55] proteomics,[56] Raf-1,[57] RASSF1A,[58] Reg IV,[59] Rsf-1,[60] Survivin,[61] Syndecan-1,[62] Synuclein-gamma,[63] TAG-72,[64] Telomeres,[65] Telomerase activity (hTERT),[66] TEM8,[67] Thrombospondin,[68] Thymidine phosphorylase,[69] Angiogenesis,[70] TNF-alpha,[71] TLRs,[72] Topoisomerase,[73] Vasculogenic mimicry,[74] VEGFs,[75-77] and XPC[78] are involved in the development or progress of GBC. Some of these directly exert an effect on clinical outcome, while some link with development or of GBC. The rapid development of molecular biology in GBC, especially in genetic diagnosis and treatment, still leave a room for scientific researchers and clinicians to seek for a much better future.

References

1. Goldin RD, Roa JC. Gallbladder cancer: A morphological and molecular update. Histopathology 2009;55:218-29.
2. Kim JH, Kim HN, Lee KT, Lee JK, Choi SH, Paik SW, *et al.* Gene expression profiles in gallbladder cancer: The close genetic similarity seen for early and advanced gallbladder cancers may explain the poor prognosis. Tumour Biol 2008;29:41-9.
3. Pandey SN, Srivastava A, Dixit M, Choudhuri G, Mittal B. Haplotype anal ysis of signal peptide (insertion/deletion) and XbaI polymorphisms of the APOB gene in gallbladder cancer. Liver Int 2007;27:1008-15.
4. Srivastava A, Tulsyan S, Pandey SN, Choudhuri G, Mittal B. Single nucleotide polymorphism in the ABCG8 transporter gene is associated with gallbladder cancer susceptibility. Liver Int 2009;29:831-7.
5. Wu K, Liao M, Liu B, Deng Z. ADAM-17 over-expression in gallbladder carcinoma correlates with poor prognosis of patients. Med Oncol 2011;28:475-80.
6. Srivastava K, Srivastava A, Mittal B. Angiotensin I-converting enzyme insertion/deletion polymorphism and increased risk of gall bladder cancer in women. DNA Cell Biol 2010;29:417-22.
7. Tan Y, Meng HP, Wu Q, Wang FQ, Wu HR. Proteomic study of gallbladder cancer, with special reference on the expression and significance of annexin A3. Zhonghua Bing Li Xue Za Zhi 2010;39:382-6.
8. Huang WY, Gao YT, Rashid A, Sakoda LC, Deng J, Shen MC, *et al.* Selected base excision repair gene polymorphisms and susceptibility to biliary tract cancer and biliary stones: A population-based case-control study in China. Carcinogenesis 2008;29:100-5.
9. Kim SY, Oh YL, Kim KM, Jeong EG, Kim MS, Yoo NJ, *et al.* Decreased expression of Bax-interacting factor-1 (Bif-1) in invasive urinary bladder and gallbladder cancers. Pathology 2008;40:553-7.
10. Puhalla H, Herberger B, Soleiman A, Filipits M, Laengle F, Gruenberger T, *et al.* E-cadherin and beta-catenin expression in normal, inflamed and cancerous gallbladder tissue. Anticancer Res 2005;25(6B):4249-54.
11. Priya TP, Kapoor VK, Krishnani N, Agrawal V, Agrawal S. Role of E-cadherin gene in gall bladder cancer and its precursor lesions. Virchows Arch 2010;456:507-14.
12. Hirata K, Ajiki T, Okazaki T, Horiuchi H, Fujita T, Kuroda Y. Frequent occurrence of abnormal E-cadherin/beta-catenin protein expression in advanced gallbladder cancers and its association with decreased apoptosis. Oncology 2006;71:102-10.

13. Adachi Y, Takeuchi T, Nagayama T, Furihata M. T-cadherin modulates tumor-associated molecules in gallbladder cancer cells. Cancer Invest 2010;28:120-6.
14. Adachi Y, Takeuchi T, Nagayama T, Ohtsuki Y, Furihata M. Zeb1-mediated T-cadherin repression increases the invasive potential of gallbladder cancer. FEBS Lett 2009;583:430-6.
15. Yin BB, Wu SJ, Zong HJ, Ma BJ, Cai D. Preliminary screening and identification of stem cell-like sphere clones in a gallbladder cancer cell line GBC-SD. J Zhejiang Univ Sci B 2011;12:256-63.
16. Shi C, Tian R, Wang M, Wang X, Jiang J, Zhang Z, *et al.* CD44+ CD133+ population exhibits cancer stem cell-like characteristics in human gallbladder carcinoma. Cancer Biol Ther 2010;10:1182-90.
17. Liu G, Ren H, Sun XJ, Shi JS. Distribution of natural killer cells and T-lymphocyte subsets in peripheral blood, gallbladder cancer and surrounding tissue. Hepatobiliary Pancreat Dis Int 2007;6:81-6.
18. Liu B, Zhang Y, Liao M, Deng Z, Gong L, Jiang J, *et al.* Clinicopathologic and prognostic significance of CD24 in gallbladder carcinoma. Pathol Oncol Res 2011;17:45-50.
19. Ai Z, Pan H, Suo T, Lv C, Wang Y, Tong S, *et al.* Arsenic oxide targets stem cell marker CD133/prominin-1 in gallbladder carcinoma. Cancer Lett 2011;310:181-7.
20. Wu W, Wang R, Liu H, Peng J, Huang D, Li B, *et al.* Prediction of prognosis in gallbladder carcinoma by CD147 and MMP-2 immunohistochemistry. Med Oncol 2009;26:117-23.
21. Li QL, Yang ZL, Liu JQ, Miao XY. Expression of CDX2 and hepatocyte antigen in benign and malignant lesions of gallbladder and its correlation with histopathologic type and clinical outcome. Pathol Oncol Res 2011;17:561-8.
22. Wani Y, Notohara K, Fujisawa M. Aberrant expression of an "intestinal marker" Cdx2 in pyloric gland adenoma of the gallbladder. Virchows Arch 2008;453:521-7.
23. Chang YT, Hsu C, Jeng YM, Chang MC, Wei SC, Wong JM. Expression of the caudal-type homeodomain transcription factor CDX2 is related to clinical outcome in biliary tract carcinoma. J Gastroenterol Hepatol 2007;22:389-94.
24. Chaube A, Tewari M, Garbyal RS, Singh U, Shukla HS. Preliminary study of p53 and c-erbB-2 expression in gallbladder cancer in Indian patients. BMC Cancer 2006;6:126.
25. Zong H, Yin B, Chen J, Ma B, Cai D, He X. Over-expression of c-FLIP confers the resistance to TRAIL-induced apoptosis on gallbladder carcinoma. Tohoku J Exp Med 2009;217:203-8.
26. Wang JW, Peng SY, Li JT, Wang Y, Zhang ZP, Cheng Y, *et al.* Identification of metastasis-associated proteins involved in gallbladder carcinoma metastasis by proteomic analysis and functional exploration of chloride intracellular channel 1. Cancer Lett 2009;281:71-81.
27. Saito S, Ghosh M, Morita K, Hirano T, Miwa M, Todoroki T. The genetic differences between gallbladder and bile duct cancer cell lines. Oncol Rep 2006;16:949-56.
28. Vishnoi M, Pandey SN, Modi DR, Kumar A, Mittal B. Genetic susceptibility of epidermal growth factor +61A>G and transforming growth factor beta1 -509C>T gene polymorphisms with gallbladder cancer. Hum Immunol 2008;69:360-7.
29. Sergeant G, Lerut E, Ectors N, Hendrickx T, Aerts R, Topal B. The prognostic relevance of tumor hypoxia markers in resected carcinoma of the gallbladder. Eur J Surg Oncol 2011;37:80-6.
30. Tewari M, Kumar V, Mishra RR, Kumar M, Shukla HS. Is there a role for cholecystectomy in gallbladder carcinoma discovered to be unresectable for cure at laparotomy? World J Surg 2008;32:2683-7.
31. Pignochino Y, Sarotto I, Peraldo-Neia C, Penachioni JY, Cavalloni G, Migliardi G, *et al.* Targeting EGFR/HER2 pathways enhances the antiproliferative effect of gemcitabine in biliary tract and gallbladder carcinomas. BMC Cancer 2010;10:631.
32. Shafizadeh N, Grenert JP, Sahai V, Kakar S. Epidermal growth factor receptor and HER-2/neu status by immunohistochemistry and fluorescence *in situ* hybridization in adenocarcinomas of the biliary tree and gallbladder. Hum Pathol 2010;41:485-92.
33. Leone F, Cavalloni G, Pignochino Y, Sarotto I, Ferraris R, Piacibello W, *et al.* Somatic mutations of epidermal growth factor receptor in bile duct and gallbladder carcinoma. Clin Cancer Res 2006;12:1680-5.
34. Hsing AW, Sakoda LC, Rashid A, Andreotti G, Chen J, Wang BS, *et al.* Variants in inflammation genes and the risk of biliary tract cancers and stones: A population-based study in China. Cancer Res 2008;68:6442-52.
35. Jia J, Li S, Gong W, Ding J, Fang C, Quan Z. mda-7/IL-24 induces apoptosis in human GBC-SD gallbladder carcinoma cells via mitochondrial apoptotic pathway. Oncol Rep 2011;25:195-201.
36. Vishnoi M, Pandey SN, Choudhuri G, Mittal B. IL-1 gene polymorphisms and genetic susceptibility of gallbladder cancer in a north Indian population. Cancer Genet Cytogenet 2008;186:63-8.
37. Lynch BC, Lathrop SL, Ye D, Ma TY, Cerilli LA. Expression of the p16(INK4a) gene product in premalignant and malignant epithelial lesions of the gallbladder. Ann Diagn Pathol 2008;12:161-4.
38. Kim WB, Han HJ, Lee HJ, Park SS, Song TJ, Kim HK, *et al.* Expression and clinical significance of cell cycle regulatory proteins in gallbladder and extrahepatic bile duct cancer. Ann Surg Oncol 2009;16:23-34.
39. Alsheyab FM, Ziadeh MT, Bani-Hani KE. Expression of p21 and p27 in gallbladder cancer. Saudi Med J 2007;28:683-7.
40. Li SH, Li CF, Sung MT, Eng HL, Hsiung CY, Huang WW, *et al.* Skp2 is an independent prognosticator of gallbladder carcinoma among p27(Kip1)-interacting cell cycle regulators: An immunohistochemical study of 62 cases by tissue microarray. Mod Pathol 2007;20:497-507.
41. Rai R, Tewari M, Kumar M, Singh AK, Shukla HS. p53: Its alteration and gallbladder cancer. Eur J Cancer Prev 2011;20:77-85.
42. Nigam P, Misra U, Negi TS, Mittal B, Choudhuri G. Alterations of p53 gene in gallbladder cancer patients of North India. Trop Gastroenterol 2010;31:96-100.
43. Castillo J, García P, Roa JC. Genetic alterations in preneoplastic and neoplastic injuries of the gallbladder. Rev Med Chil 2010;138:595-604.
44. Wang SN, Chung SC, Tsai KB, Chai CY, Chang WT, Kuo KK, *et al*. Aberrant p53 expression and the development of gallbladder carcinoma and adenoma. Kaohsiung J Med Sci 2006;22:53-9.
45. Arévalo F, Arias Stella C, Monge E. Immunoexpression of p53 and cyclin D1 in adenomas of the gallbladder. Rev Esp Enferm Dig 2007;99:694-7.
46. Nagahashi M, Ajioka Y, Lang I, Szentirmay Z, Kasler M, Nakadaira H, *et al*. Genetic changes of p53, K-ras, and microsatellite instability in gallbladder carcinoma in high-incidence areas of Japan and Hungary. World J Gastroenterol 2008;14:70-5.
47. Takagawa M, Muguruma N, Oguri K, Imoto Y, Okamoto K,

Ii K, *et al.* Prediction of prognosis in gallbladder carcinoma by mucin and p53 immunohistochemistry. Dig Dis Sci 2005;50:1410-3.
48. Chang HJ, Yoo BC, Kim SW, Lee BL, Kim WH. Significance of PML and p53 protein as molecular prognostic markers of gallbladder carcinomas. Pathol Oncol Res 2007;13:326-35.
49. Mishra PK, Jatawa SK, Raghuram GV, Pathak N, Jain A, Tiwari A, *et al.* Correlation of aberrant expression of p53, Rad50, and cyclin-E proteins with microsatellite instability in gallbladder adenocarcinomas. Genet Mol Res 2000;8:1202-10.
50. Yanagisawa N, Yamashita K, Kuba T, Okayasu I. Sporadic TP53 transition mutations in chronic cholecystitis are possibly linked to gallbladder carcinogenesis. Anticancer Res 2010;30:4443-9.
51. Moreno M, Pimentel F, Gazdar AF, Wistuba II, Miquel JF. TP53 abnormalities are frequent and early events in the sequential pathogenesis of gallbladder carcinoma. Ann Hepatol 2005;4:192-9.
52. Deshpande V, Nduaguba A, Zimmerman SM, Kehoe SM, Macconaill LE, Lauwers GY, *et al.* Mutational profiling reveals PIK3CA mutations in gallbladder carcinoma. BMC Cancer 2011;11:60.
53. García P, Manterola C, Araya JC, Villaseca M, Guzmán P, Sanhueza A, *et al.* Promoter methylation profile in preneoplastic and neoplastic gallbladder lesions. Mol Carcinog 2009;48:79-89.
54. Roa JC, Anabalón L, Roa I, Melo A, Araya JC, Tapia O, *et al.* Promoter methylation profile in gallbladder cancer. J Gastroenterol 2006;41:269-75.
55. Lee YM, Lee JY, Kim MJ, Bae HI, Park JY, Kim SG, *et al.* Hypomethylation of the protein gene product 9.5 promoter region in gallbladder cancer and its relationship with clinicopathological features. Cancer Sci 2006;97:1205-10.
56. Tan Y, Meng HP, Wang FQ, Cheng ZN, Wu Q, Wu HR. Comparative proteomic analysis of human gallbladder carcinoma. Zhonghua Zhong Liu Za Zhi 2010;32:29-32.
57. Kim HS, Kim GY, Lim SJ, Park YK, Kim YW. Reduced expression of Raf-1 kinase inhibitory protein is a significant prognostic marker in patients with gallbladder carcinoma. Hum Pathol 2010;41:1609-16.
58. Kee SK, Lee JY, Kim MJ, Lee SM, Jung YW, Kim YJ, *et al.* Hypermethylation of the Ras association domain family 1A (RASSF1A) gene in gallbladder cancer. Mol Cells 2007;24:364-71.
59. Tamura H, Ohtsuka M, Washiro M, Kimura F, Shimizu H, Yoshidome H, *et al.* Reg IV expression and clinicopathologic features of gallbladder carcinoma. Hum Pathol 2009;40:1686-92.
60. Chen TJ, Huang SC, Huang HY, Wei YC, Li CF. Rsf-1/HBXAP overexpression is associated with disease-specific survival of patients with gallbladder carcinoma. APMIS 2011;119:808-14.
61. Liu C, Sun B, An N, Tan W, Cao L, Luo X, *et al.* Inhibitory effect of survivin promoter-regulated oncolytic adenovirus carrying p53 gene against gallbladder cancer. Mol Oncol 2011;5:545-54.
62. Roh YH, Kim YH, Choi HJ, Lee KE, Roh MS. Syndecan-1 expression in gallbladder cancer and its prognostic significance. Eur Surg Res 2008;41:245-50.
63. Han S, She F, Wang D, Yao X, Jiang L, Chen Y. SNCG gene silencing in gallbladder cancer cells inhibits key tumorigenic activities. Front Biosci 2012;17:1589-98.
64. Ouyang M, Wu W, Zou Y, Zhou J, Wang Z, Wan X. Immunoreactivity and prognostic value of tumor-associated glycoprotein 72 in primary gallbladder carcinoma. Surg Oncol 2010;19:82-7.
65. Hansel DE, Meeker AK, Hicks J, De Marzo AM, Lillemoe KD, Schulick R, *et al.* Telomere length variation in biliary tract metaplasia, dysplasia, and carcinoma. Mod Pathol 2006;19:772-9.
66. Shukla VK, Chauhan VS, Kumar M. Telomerase activation – one step on the road to carcinoma of the gall bladder. Anticancer Res 2006;26(6C):4761-6.
67. Maurya SK, Tewari M, Kumar M, Thakur MK, Shukla HS. Expression pattern of tumor endothelial marker 8 protein in gallbladder carcinomas. Asian Pac J Cancer Prev 2011;12:507-12.
68. Roh YH, Kim YH, Choi HJ, Lee KE, Roh MS. Fascin overexpression correlates with positive thrombospondin-1 and syndecan-1 expressions and a more aggressive clinical course in patients with gallbladder cancer. J Hepatobiliary Pancreat Surg 2009;16:315-21.
69. Won HS, Lee MA, Chung ES, Kim DG, You YK, Hong TH, *et al.* Comparison of thymidine phosphorylase expression and prognostic factors in gallbladder and bile duct cancer. BMC Cancer 2010;10:564.
70. Harino Y, Imura S, Kanemura H, Morine Y, Fujii M, Ikegami T, *et al.* Role of tumor angiogenesis in gallbladder carcinoma: With special reference to thymidine phosphorylase. Int J Clin Oncol 2008;13:452-7.
71. Vishnoi M, Pandey SN, Choudhury G, Kumar A, Modi DR, Mittal B. Do TNFA -308G/A and IL6-174G/C gene polymorphisms modulate risk of gallbladder cancer in the north Indian population? Asian Pac J Cancer Prev 2007;8:567-72.
72. Srivastava K, Srivastava A, Kumar A, Mittal B. Significant association between toll-like receptor gene polymorphisms and gallbladder cancer. Liver Int 2010;30:1067-72.
73. Washiro M, Ohtsuka M, Kimura F, Shimizu H, Yoshidome H, Sugimoto T, *et al.* Upregulation of topoisomerase IIalpha expression in advanced gallbladder carcinoma: A potential chemotherapeutic target. J Cancer Res Clin Oncol 2008;134:793-801.
74. Sun W, Fan YZ, Zhang WZ, Ge CY. A pilot histomorphology and hemodynamic of vasculogenic mimicry in gallbladder carcinomas *in vivo* and *in vitro*. J Exp Clin Cancer Res 2011;30:46.
75. Chen Y, Jiang L, She F, Tang N, Wang X, Li X, *et al.* Vascular endothelial growth factor-C promotes the growth and invasion of gallbladder cancer via an autocrine mechanism. Mol Cell Biochem 2010;345:77-89.
76. Jiang L, Chen YL, She FF, Tang NH, Li XJ, Wang XX. Expressions of VEGF-C and VEGF-D and their correlation with lymphangiogenesis and angiogenesis in gallbladder carcinoma. Zhonghua Zhong Liu Za Zhi 2010;32:190-5.
77. Lin W, Jiang L, Chen Y, She F, Han S, Zhu J, *et al.* Vascular endothelial growth factor-D promotes growth, lymphangiogenesis and lymphatic metastasis in gallbladder cancer. Cancer Lett 2012;314:127-36.
78. Jiao X, Ren J, Chen H, Ma J, Rao S, Huang K, *et al.* Ala499Val (C>T) and Lys939Gln (A>C) polymorphisms of the XPC gene: Their correlation with the risk of primary gallbladder adenocarcinoma – a case-control study in China. Carcinogenesis 2011;32:496-501.

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