Retinoid X receptor α enhances human cholangiocarcinoma growth through simultaneous activation of Wnt/β-catenin and nuclear factor-κB pathways

Gui-Li Huang,1 Wei Zhang,2 Hong-Yue Ren,3 Xue-Ying Shen,1 Qing-Xi Chen1 and Dong-Yan Shen3

1State Key Laboratory of Cellular Stress Biology, School of Life Sciences, Xiamen University, Xiamen; Divisions of 2 Xiamen Diabetes Institute; 3Biobank, The First Affiliated Hospital of Xiamen University, Xiamen, China

Key words
Cholangiocarcinoma, NF-κB, proliferation, retinoid X receptor α, β-catenin

Correspondence
Dong-Yan Shen, No. 55, Road of Zhenhai, Siming Zone, Xiamen 361003, China.
Tel: +86-592-2137507; Fax: +86-592-2137509; E-mail: shendongyang@163.com
Qing-Xi Chen, School of Life Sciences, Xiamen University, Xiamen 361102, China.
Tel: +86-592-2185487; Fax: +86-592-2185487; E-mail: chenqx@xmu.edu.cn

Funding Information
National Natural Science Foundation of China (81572394 and 81470544); the National Science Foundation for Fostering Talents in Basic Research of the National Natural Science Foundation of China (J1310027).

Received April 7, 2015; Revised August 10, 2015; Accepted August 22, 2015

Cancer Sci 106 (2015) 1515–1523

doi: 10.1111/cas.12802

Cholangiocarcinoma (CCA) is the second most frequent primary hepatic malignancy, originating from biliary epithelial cells. Recent studies showed that the morbidity and mortality of CCA has been increasing all over the world during the past three decades.1) Prognosis of CCA is poor, with a 5-year survival rate <5%.2,3) Due to insufficiency of conventional chemotherapy and radiotherapy in improving long-term survival, surgical resection is the only effective therapy for early stage tumors.4) Therefore, it is urgent to clarify the molecular mechanisms underlying CCA proliferation for development of novel effective therapeutic targets.

The retinoid X receptors (RXRs) are members of the steroid/thyroid hormone superfamily of nuclear receptors, which are transcription factors that are essential in embryonic development, maintenance of differentiated phenotypes, metabolism, and cell death.5,6) There are three RXR subtypes, α, β, and γ. Among them, RXRα plays unique and uncharacterized roles in many physiological processes including carcinogenesis. Previous studies showed that RXRα is overexpressed in multiple human cancers such as human prostate tumor,7) breast cancer,8) and thyroid tumor.9) Retinoid X receptor α (RXRα) plays important roles in the malignancy of several cancers such as human prostate tumor, breast cancer, and thyroid tumor. However, its exact functions and molecular mechanisms in cholangiocarcinoma (CCA), a chemoresistant carcinoma with poor prognosis, remain unclear. In this study we found that RXRα was frequently overexpressed in human CCA tissues and CCA cell lines. Downregulation of RXRα led to decreased expression of mitosis-promoting factors including cyclin D1 and cyclin E, and the proliferating cell nuclear antigen, as well as increased expression of cell cycle inhibitor p21, resulting in inhibition of CCA cell proliferation. Furthermore, RXRα knockdown attenuated the expression of cyclin D1 through suppression of Wnt/β-catenin signaling. Retinoid X receptor α upregulated proliferating cell nuclear antigen expression through nuclear factor-κB (NF-κB) pathways, paralleled with downregulation of p21. Thus, the Wnt/β-catenin and NF-κB pathways account for the inhibition of CCA cell growth induced by RXRα downregulation. Retinoid X receptor α plays an important role in proliferation of CCA through simultaneous activation of Wnt/β-catenin and NF-κB pathways, indicating that RXRα might serve as a potential molecular target for CCA treatment.

In the present study, we show that the expression of RXRα is frequently elevated in human CCA specimens and CCA cell lines. Knockdown of RXRα in CCA cells resulted in remarkable suppression of Wnt/β-catenin and nuclear factor-κB (NF-κB) pathways, leading to inhibition of cell proliferation. Our findings might be helpful for understanding the role of RXRα in the development of CCA.

© 2015 The Authors. Cancer Science published by Wiley Publishing Asia Pty Ltd on behalf of Japanese Cancer Association.
This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
Materials and Methods

Reagents and antibodies. Wnt3a, BMS345541, DAPI, and MTT were from Sigma-Aldrich (Indianapolis, IN, USA). Fetal calf serum and RPMI-1640 medium were purchased from Gibco (Grand Island, NY, USA). Lipofectamine RNAi Max, Lipofectamine 2000, stealth-siRNA, goat anti-mouse, and anti-rabbit secondary antibodies conjugated to HRP, and donkey anti-rabbit antibody–Alexa Fluor 467 were purchased from Invitrogen (Carlsbad, CA, USA). Monoclonal antibodies against cyclin A, cyclin B1, cyclin D1, cyclin E, p21, β-actin, and polyclonal antibody against RXRα were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Polyclonal antibodies against β-catenin and phosphorylated β-catenin were purchased from Cell Signaling Technology (Danvers, MA, USA). The PVDF membranes were obtained from Millipore (Billerica, MA, USA) and the 5-ethyl-2'-deoxyuridine (EdU) assay kit was from RiboBio (Guangzhou, China). The Dual-Glo Luciferase Assay System kit was purchased from Promega (Madison, WI, USA) and the EliVision Plus kit was from Maixin Bio (Fuzhou, China).

Patients and tumor specimens. Tumorous and their adjacent non-cancerous CCA tissues were collected from 54 patients who underwent surgery at the First Affiliated Hospital of Xiamen University (Xiamen, China). Written informed consent was obtained from each patient and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institute Research Ethics Committee of the First Affiliated Hospital of Xiamen University. Fresh surgical samples from CCA tissues were collected between 2008 and 2013.

Cell culture and transfection. QBC939 cells were obtained from Shuguang Wang (Third Military Medical University, Chongqing China). SK-ChA-1 and MZ-ChA-1 cells were kindly provided by Dr. Yabin Chen (University of Alabama at Birmingham, Birmingham, AL, USA). The above three cell lines were previously published. HCC9810 and human intrahepatic biliary epithelial cells (HIBEpiC) were purchased from Cell Bank of the Chinese Academy of Sciences (Beijing, China). QBC939, MZ-ChA-1, SK-ChA-1, and HCC9810 human cholangiocarcinoma cells and HIBEpiC human intrahepatic biliary epithelial cells were cultured in RPMI-1640 supplemented with 10% FBS, 100 U/mL penicillin, and 100 U/mL streptomycin at 37 °C in an atmosphere of 5% CO2. Stealth siRNA (40 nM) targeting RXRα (Invitrogen) and non-specific Stealth siRNA (siCtrl) (40 nM) were transfected with Lipofectamine RNAi Max (Invitrogen) according to the manufacturer’s instructions. Myc-RXRα and pCMV-myc (Ctrl) were transfected with Lipofectamine 2000 according to the manufacturer’s instructions.

Cell viability assay. Cells were seeded in 96-well plates and MTT was added to each well. The plates were incubated at 37 °C for 4 h, followed by addition of 100 µL DMSO. The absorbance was measured at 490 nm using a microplate reader (Model 680; Bio-Rad, Hercules, CA, USA).

Colony formation. Five hundred cells were cultured in 6-well plates for 2 weeks. The colonies were fixed and stained with 0.005% crystal violet for 30 min and counted.

Cell proliferation assay. 5-Ethynyl-2'-deoxyuridine is a nucleoside analog of thymidine and is incorporated into DNA during active DNA synthesis. Cells were seeded in each well of a 96-well plate, and then transfected with siRNA (40 nM) and siCtrl (40 nM) using Lipofectamine RNAi Max after 24 h. The cells were incubated for 2 h after the addition of 100 µL

EdU solution (50 µM), then stained with Apollo. Finally, Hoechst 33342 solution was added to visualize the nuclei. The staining was examined using a Leica TCS SP5 II laser confocal microscope (Leica, Barcelona, Spain).

Immunohistochemistry. Paraffin-embedded human CCA tissue sections were pretreated with blocking buffer (5% normal goat serum in PBS) for 30 min at room temperature, and then immunostained with antibody against RXRα (1:200) at 4 °C overnight, followed by incubation with secondary antibody conjugated with HRP. Images were collected and analyzed using an inverted fluorescence microscope. The staining intensity of RXRα protein was categorized into four different grades according to their different positive rates. The staining intensity ± and + were considered as low expression, and ++ and +++ were considered as high expression.

Immunofluorescence. Cells were seeded on glass slides overnight. After siRXR or siCtrl (40 nM) transfection for 24 h, and then Wnt3a (50 ng/mL) treatment for 6 h, cells were fixed with 4% paraformaldehyde for 15 min, permeabilized using 0.5% Triton X-100 for 20 min, and blocked using normal donkey serum for 30 min. The primary antibodies (β-catenin, 1:200) were added and incubated at 4 °C overnight. The slides were washed and incubated with Alexa Fluor 467-conjugated secondary antibodies (1:100) at room temperature for 30 min, then DAPI (1 µg/mL) was used to stain nuclei. The images were taken under a Leica TCS SP5 II laser confocal microscope using the LSM-510 confocal laser scanning microscope system, as previously described.(17)

Quantitative PCR. Total RNA was extracted using a Simple RNA Extraction kit (Tiangen, Beijing, China) according to the manufacturer’s instructions. Reverse transcription was carried out using the SuperScript III First-Strand Synthesis System (Invitrogen) for real-time RT-PCR. The RT-PCR was carried out in 96-well plates with the ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Relative quantification was analyzed by normalization to the amount of human GAPDH. Primers used for real-time PCR are listed on Table 1.

Western blot analysis. The concentration of extracted proteins was determined with the BCA Kit (Thermo Scientific

Table 1. Primer sequences for quantitative PCR study of genes involved in cholangiocarcinoma

| Gene      | Primer sequence (5′–3′)               |
|-----------|--------------------------------------|
| GAPDH     | Sense GAACACTTCTTGATCTGCTGTTGGT       |
|           | Antisense TACGTTAAGTGGCCTGT           |
| RXRα      | Sense ATCACCTTTCTGGCCATCA             |
|           | Antisense TAACGATTTGCTGCTG            |
| Cyclin A  | Sense CCGATGTCACGAGGAACTGCAA          |
|           | Antisense TGGTCTTGGCTGCTG             |
| Cyclin B1 | Sense CCTCAACCACCTTGCTGGC            |
|           | Antisense AAGGATGCCTTGACG             |
| Cyclin D1 | Sense AAGGATCCCTTGCTGG               |
|           | Antisense CATTTCTGAGCAACCC            |
| Cyclin E  | Sense ATTTCTCAAGTTTGCTGCA             |
|           | Antisense GGCCAGAGCTTCCCCTTACG        |
| P21       | Sense CAGGATCTCCTTGCTGCG             |
|           | Antisense TAGCTGTTCGGCTTCGAGG         |
| PCNA      | Sense TAGCTGTTCGGCTTCGAGG             |

© 2015 The Authors. Cancer Science published by Wiley Publishing Asia Pty Ltd on behalf of Japanese Cancer Association.
Western blot analysis was carried out as previously described. Equal amounts of protein lysates underwent 10% SDS-PAGE and then were transferred onto a PVDF membrane (Millipore). The membrane was incubated with primary antibodies, followed by incubation with secondary antibody-conjugated HRP and the signal was finally visualized by chemiluminescence (Tiangen).

**Flow cytometry.** QBC939 cells treated by siRNA (Ctrl or RXRα) for 24 h were harvested, and fixed in 75% ethanol at 4°C overnight. Cells were incubated with RNase A at 37°C for 30 min, and then stained with propidium iodide (Sigma-Aldrich). Cell cycle was measured by flow cytometry. The data were analyzed with the ModFit 3.3 (Verity Software House, Topsham, ME, USA) software.

**Dual-luciferase reporter assay.** QBC939, MZ-ChA-1, or HCCC9810 cells (1.0 × 10^4 cells/well) were seeded in 96-well plates for 24 h before transfection. QBC939 and MZ-ChA-1 cells were transfected with siRNA (RXRα or Ctrl) for 24 h. HCCC9810 cells were transfected with Myc-RXRα and pCMV-myc (Ctrl) for 24 h. Then cells in each well were cotransfected with 100 ng pTOPFlash or pFOPFlash or p65 reporter plasmid and 20 ng Renilla luciferase expression vector using Lipofectamine 2000 for another 24 h and subsequently with Wnt3a (50 ng/mL) for 6 h or tumor necrosis factor α (TNFα; 10 ng/mL) for 4 h. The indicated cells were analyzed for luciferase activity by using a Dual-Glo Luciferase Assay System (Promega), according to the manufacturer’s instruc-

---

**Table 2. Clinicopathological characteristics of patients with cholangiocarcinoma (n = 54)**

| Feature                  | n (%) |
|--------------------------|-------|
| Patients enrolled        | 54    |
| Age, years               |       |
| Median                   | 58    |
| Range                    |       |
| <50                      | 11 (20.4) |
| ≥50                      | 43 (79.6) |
| Sex                      |       |
| Male                     | 45 (83.3) |
| Female                   | 9 (16.7) |
| Tumor differentiation    |       |
| MP + P                   | 28 (51.8) |
| M                        | 17 (31.5) |
| W + WM                   | 9 (16.7) |
| Lymph node status        |       |
| Node-negative            | 19 (35.2) |
| Node-positive            | 35 (64.8) |
| RXRα expression          |       |
| High                     | 37 (68.5) |
| Low                      | 17 (31.5) |

M, moderately differentiated; MP, moderately to poorly differentiated; P, poorly differentiated; RXRα, retinoid X receptor α; W, well differentiated; WM, well to moderately differentiated.
Statistical analysis. Statistical analysis was carried out using GraphPad Prism 6 (San Diego, CA, USA). The results were expressed as the mean ± SD. The data obtained from at least three independent experiments. Student’s t-test and one-way ANOVA were used for comparison of two or more datasets, respectively. P-value <0.05 was considered statistically significant.
Results

Retinoid X receptor α (RXRα) overexpressed in human CCA tissues and cell lines. To evaluate the expression of RXRα in CCA, immunohistochemistry was carried out to assess RXRα protein levels in a set of 54 tumor tissues and their surrounding non-cancerous tissues. The results confirmed that RXRα protein was predominantly present in the cell cytoplasm (Fig. 1a). The high expression rate of RXRα protein in CCA tissues was 68.5% (37/54) (Table 2). Furthermore, RXRα protein predominantly existed in the cytoplasm of CCA cell line QBC939, as shown by the immunofluorescence (IF) results (Fig. 1b). In addition, the expression of RXRα was significantly increased in CCA cell lines such as QBC939, MZ-ChA-1, and SK-ChA-1 compared with HIBEpiC (Fig. 1c). These data showed that RXRα is markedly overexpressed in CCA patients as well as in CCA cell lines.

Retinoid X receptor α knockdown inhibits CCA cell proliferation. To investigate the role of RXRα in the growth of CCA, QBC939, MZ-ChA-1, and SK-ChA-1 cells, in which elevated RXRα protein level was observed, were transfected with siRXRα or siCtrl. HCCC9810 cells with low levels of RXRα were transfected with RXRα-expression vector. Downregulation of RXRα in CCA cells decreased cell survival compared with their respective controls, whereas overexpression of RXRα in HCCC9810 cells increased cell survival (Fig. 2a). Furthermore, the ability of QBC939, MZ-ChA-1, and SK-ChA-1 cells to form foci were markedly reduced after transfection with siRXRα in contrast to that with siCtrl, whereas the ability of HCCC9810 cells to form foci was significantly increased with RXRα overexpression compared to control (Fig. 2b). The EdU assay was then applied to analyze cell proliferation. Cell proliferation was significantly decreased in siRXRα-QBC939 cells, and significantly increased in RXRα-overexpressed HCCC9810 cells (Fig. 2c). Together, these data indicated that RXRα expression contributes to CCA cell proliferation.

Retinoid X receptor α knockdown induces CCA cell cycle arrest. To investigate the potential mechanism by which downregula-
tion of RXRα inhibited proliferation of CCA cells, cell cycle analysis was carried out to examine whether RXRα knocked-down cells are arrested in a specific phase of the cell cycle. Flow cytometry results showed that RXRα knockdown induced G1 arrest in QBC939 cells (Fig. 3a). Quantitative PCR and Western blotting results showed that cyclin D1 and cyclin E were decreased and p21 was increased in CCA cells transfected with siRXRα compared with siCtrl cells, whereas the expression of cyclin A1 and cyclin B1 was comparable (Fig. 3b, c). In contrast, overexpression of RXRα in HCCC9810 cells strongly increased cyclin D1 and cyclin E expression and significantly decreased p21 expression, whereas the other cycle-related proteins were also comparable (Fig. 3b, d). Furthermore, the proliferation marker proliferating cell nuclear antigen (PCNA) was upregulated by RXRα overexpression, and was downregulated by RXRα knockdown in CCA cells. These results suggested that the expression of RXRα could promote cell cycle progression.

Retinoid X receptor α knockdown inhibits activation of Wnt/β-catenin pathway. Cyclin D1, the downstream target gene of the Wnt/β-catenin signaling pathway, was regulated by RXRα (Fig. 3b, c). Thus, we investigated whether RXRα affected the regulation of the Wnt/β-catenin signaling pathways in CCA. Western blot analysis showed that the expression of β-catenin was decreased in RXRα knockdown CCA cells, along with phosphorylation of β-catenin at ser33/37 was increased. Furthermore, Wnt3a-induced accumulation of β-catenin slightly decreased after transfection of RXRα siRNA (Fig. 4a). In contrast, Wnt3a significantly increased total β-catenin, and strongly suppressed phosphorylation and degradation of β-catenin, which was strengthened by overexpression of RXRα (Fig. 4a). Immunofluorescence results revealed that Wnt3a-induced expression of β-catenin was suppressed by RXRα knockdown, which was in line with the above Western blotting results (Fig. 4b). Dual-luciferase reporter assays showed that RXRα knockdown significantly reduced Wnt reporter activity. In contrast, overexpression of RXRα enhanced Wnt reporter activity, which was further increased in the treatment of Wnt3a (Fig. 4c). In addition, we examined whether β-catenin knockdown downregulated cyclin D1 expression.
expression in CCA cells. As shown in Fig. 4(d), downregulation of β-catenin led to decrease of cyclin D1, which was not rescued by treatment with Wnt3a. Overexpression of RXRa increased cyclin D1 expression in HCCC9810 cells, which was cancelled by β-catenin knockdown (Fig. 4e). Collectively, these results indicated that RXRa promoted CCA cell cycle progression, at least in part, through activation of the Wnt/β-catenin signaling pathway.

Retinoid X receptor α regulates PCNA and p21 expression through activation of NF-κB signaling. We reported previously that PCNA and p21 were the targets of NF-κB pathway in CCA cells. We therefore investigated whether RXRa regulated PCNA and p21 through activation of the NF-κB pathway. Treatment of CCA cells with TNFα led to strong p65 activation, which was potently impaired by RXRa knockdown; phosphorylation of p65 at ser536 was strongly activated by TNFα, which was enhanced in RXRa-overexpressed cells (Fig. 5a). Dual-luciferase reporter assays revealed that p65 reporter activity was significantly enhanced by TNFα and was suppressed by RXRa knockdown. Furthermore, activation of p65 reporter by treatment with TNFα was reduced by RXRa knockdown (Fig. 5b). The expression of p21 was downregulated and PCNA was upregulated in the RXRa-overexpressed cells, and was reversed by BMS345541, a specific inhibitor of NF-κB (Fig. 5c). Taken together, these results showed that RXRa might serve as a cofactor for the transduction of the NF-κB signaling pathway for regulation of PCNA and p21.

Discussion

Retinoid X receptor α plays vital genomic roles through serving as an obligatory DNA-binding partner for a number of nuclear receptors. It also shows non-genomic regulatory effects, similar to retinoic acid receptor γ, as we reported previously. In the present study, we found that RXRa protein expression was significantly increased and was abnormally expressed in the cytoplasm of human CCA specimens, in agreement with previous findings that RXRa resides in the cytoplasm in certain cell types. Overexpression of RXRa was also detected in all CCA cell lines examined in the present study. Paradoxically, RXRa expression was significantly suppressed in colorectal cancer compared with adjacent non-tumor tissues. Our results concurred with previous studies regarding the expression profile of RXRa in breast cancer, prostate tumor, and thyroid tumor, indicating that the cytoplasmic localization of RXRa may be in a cell type-dependent manner. The detailed mechanisms for RXRa overexpression in CCA remain unclear.
Retinoid X receptor α has been implicated in several neoplastic diseases. The most fundamental trait of cancer cells is the ability to sustain chronic proliferation through the cell cycle.\(^{(27)}\) Our data presented here showed that down-regulation of RXRα repressed CCA cell proliferation, colony formation, and DNA synthesis in CCA cells. Knockdown of RXRα resulted in cell cycle arrest at G1 phase through reducing the expression of cyclin D1 and cyclin E, two critical S transition regulators,\(^{(28)}\) paralleled with upregulation of p21, the cell cycle inhibitor.\(^{(29)}\) In contrast, RXRα overexpression increased cyclin D1 and cyclin E expression, and suppressed p21 expression. Furthermore, RXRα upregulated the expression of the proliferation marker PCNA.\(^{(30)}\) This indicates its important role in the growth of human CCA. These results suggested a key role of RXRα in CCA proliferation.

The present study has, for the first time, explored the possible mechanisms of RXRα on regulation of CCA cell growth. We showed that RXRα activated the Wnt/β-catenin and NF-κB survival pathways to induce CCA cell growth (Fig. 5d). It has been shown that many oncogetic factors lead to aberrant activation of the Wnt/β-catenin pathway in many types of oncogetic diseases, including CCA.\(^{(31)}\) In the Wnt-stimulated cell, β-catenin is not targeted for degradation in the ubiquitin proteasome. Instead, it is transported to the nuclei, where it interacts with lymphoid enhancing factor/T-cell factor transcription factors to alter target gene expression so as to regulate cell proliferation and cell cycle progression.\(^{(32)}\) The activation of Wnt/β-catenin signaling contributes to cell proliferation through upregulation of the vital cell cycle regulator cyclin D1, which was confirmed by our previous work.\(^{(33)}\) In addition, our findings revealed that RXRα knockdown significantly reduced total β-catenin expression and increased phosphorylation of β-catenin; however, there were reverse trends of β-catenin in RXRα-overexpressed cells, indicating that RXRα might promote CCA growth through activation of the Wnt/β-catenin pathway. Our results were in agreement with the study by Xiao et al.,\(^{(34)}\) which concluded that β-catenin interacts with RXRα in cancer cells, and RXR agonists, which degrade RXRα, induce degradation of β-catenin.

Our view that the oncogenic effects of RXRα can be attributed to activation of the NF-κB signaling pathway provides insight into the link between inflammation-mediated cell signaling and regulation of RXRα. Previous studies showed that NF-κB p65 directly interacts with the DNA-binding domain of RXRα.\(^{(35)}\) Hence, it is not surprising to show that RXRα could coactivate NF-κB transcription factor during human CCA development. Our study revealed that RXRα knockdown could regulate the expression of PCNA and p21 though suppression of the NF-κB pathway. These results suggested that the activation of the Wnt/β-catenin and NF-κB pathways is essential for RXRα-mediated cell cycle progression in CCA cells.

In conclusion, our study showed that RXRα is able to simultaneously activate the Wnt/β-catenin and NF-κB pathways to promote CCA cell proliferation and survival. Thus, RXRα might be an attractive molecular target for drug development in treatment of CCA. Our findings are potentially beneficial for the future development of novel therapeutic approaches against CCA.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant Nos. 81572394 and 81470544), the National Science Foundation for Fostering Talents in Basic Research of the National Natural Science Foundation of China (Grant No. J1310027), the Science and Technology Foundation of Xiamen, China (Grant Nos. 3502ZZ20144002 and 350220133009), and Project of Young and Middle-aged Backbone Talents Cultivation, Fujian, China (Grant No. 2015-ZQN-ZD31).

Disclosure Statement

The authors have no conflict of interest.

References

1. Zabron A, Edwards RJ, Khan SA. The challenge of cholangiocarcinoma: dissecting the molecular mechanisms of an insidious cancer. Dis Model Mech 2013; 6: 81–92.
2. Lazaridis KN, Gores GJ. Cholangiocarcinoma. Gastroenterology 2005; 128: 1655–67.
3. Ramia JM. Hilar cholangiocarcinoma. World J Gastrointest Oncol 2013; 5: 113–4.
4. Ramirez-Merino N, Ais SP, Cortes-Funes H. Chemotherapy for cholangiocarcinoma: an update. World J Gastrointest Oncol 2013; 5: 171–6.
5. Gronemeier H, Gustafsson J-A, Laudet V. Principles for modulation of the nuclear receptor superfamily. Nat Rev Drug Discovery 2004; 3: 950–6.
6. Maire A, Alvarez S, Shankaranarayanan P, Lera AR, Bourguet W, Gronemeier H. Retinoid receptors and therapeutic applications ofRAR/RXR modulators. Curr Top Med Chem 2012; 12: 505–27.
7. Zhong C, Yang S, Huang J, Cohen MB, Roy-Kurman P. Aberration in the expression of the retinoid receptor, RXRalpha, in prostate cancer. Cancer Biol Ther 2003; 2: 179–84.
8. Crowe DL, Chandraratna RA. A retinoid X receptor (RXR)-selective retinoid reveals that RXR-alpha is potentially a therapeutic target in breast cancer cell lines, and that it potentiates antiproliferative and apoptotic responses to peroxisome proliferator-activated receptor ligands. Breast Cancer Res 2004; 6: R56–63.
9. Takiyama Y, Miyokawa N, Sugawara A et al. Decreased expression of retinoid X receptor isoforms in human thyroid carcinomas. J Clin Endocrinol Metab 2004; 89: 5851–61.
10. Miyazaki S, Taniguchi H, Morito Y et al. Nuclear hormone retinoid X receptor (RXR) negatively regulates the glucose-stimulated insulin secretion of pancreatic β-cells. Diabetes 2010; 59: 2854–61.
11. Altucci L, Gronemeyer H. The promise of retinoids to fight against cancer. Nat Rev Cancer 2001; 1: 181–93.
12. Zhu J, Naor R, Pérez L et al. RXR is an essential component of the oncogetic PML/RARA complex in vivo. Cancer Cell 2007; 12: 25–35.
13. Dawson MI, Zhang X-K. Discovery and design of retinoic acid receptor and retinoid X receptor class- and subtype-selective synthetic analogs of all-trans-retinoic acid and 9-cis-retinoic acid. Curr Med Chem 2002; 9: 623–37.
14. Kempf W, Kettelhack N, Duvic M, Burg G. Topical and systemic retinoid therapy for cutaneous T-cell lymphoma. Hematol Oncol Clin North Am 2003; 17: 1405–19.
15. Zhang C, Duvic M. Retinoids: therapeutic applications and mechanisms of action in cutaneous T-cell lymphoma. Dermatol Ther 2003; 16: 322–30.
16. Chen Q, Li W, Wan Y et al. Amplified in breast cancer 1 enhances human cholangiocarcinoma growth and chemoresistance by simultaneous activation of Akt and Nrf2 pathways. Hepatology 2012; 55: 1820–9.
17. Huang GL, Luo Q, Rui G et al. Oncogenic activity of retinoic acid receptor gamma is exhibited through activation of the Akt/NF-kappaB and Wnt/beta-catenin pathways in cholangiocarcinoma. Mol Cell Biol 2013; 33: 3416–25.
18. Shen DY, Fang ZX, You P et al. Clinical significance and expression of cyclin kinase subunits 1 and 2 in hepatocellular carcinoma. Liver Int 2010; 30: 119–25.
19. Chikazawa K, Tanaka H, Tasaka T et al. Inhibition of Wnt signaling pathway decreases chemotherapy-resistant side-population colon cancer cells. Anticancer Res 2010; 30: 2041–8.
20. Flahaut M, Meier R, Coulon A et al. The Wnt receptor FZD1 mediates chemoresistance in neuroblastoma through activation of the Wnt/beta-catenin pathway. Oncogene 2009; 28: 2245–56.
21. Sottmann M, Zbarinsky J, Simcha I et al. The cyclin D1 gene is a target of the beta-catenin/LEF-1 pathway. Proc Natl Acad Sci U S A 1999; 96: 5522–7.
22 Zhang H, Zhang X, Wu X et al. Interference of Frizzled 1 (FZD1) reverses multidrug resistance in breast cancer cells through the Wnt/beta-catenin pathway. *Cancer Lett* 2012; 323: 106–13.
23 Perez E, Bourguet W, Gronemeyer H, de Lera AR. Modulation of RXR function through ligand design. *Biochim Biophys Acta* 2012; 1821: 57–69.
24 Fukunaka K, Saito T, Wataba K, Ashihara K, Ito E, Kudo R. Changes in expression and subcellular localization of nuclear retinoic acid receptors in human endometrial epithelium during the menstrual cycle. *Mol Hum Reprod* 2001; 7: 437–46.
25 Zhang F, Meng F, Li H, Dong Y, Yang W, Han A. Suppression of retinoid X receptor alpha and aberrant beta-catenin expression significantly associates with progression of colorectal carcinoma. *Eur J Cancer* 2011; 47: 2060–7.
26 Lawrence JA, Merino MJ, Simpson JF, Manrow RE, Page DL, Steeg PS. A high-risk lesion for invasive breast cancer, ductal carcinoma in situ, exhibits frequent overexpression of retinoid X receptor. *Cancer Epidemiol Biomarkers Prev* 1998; 7: 29–35.
27 Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144: 646–74.
28 Hahnvajanawong C, Ketnimit S, Pattanapanyasat K et al. Involvement of p53 and nuclear factor-kappaB signaling pathway for the induction of G1-phase cell cycle arrest of cholangiocarcinoma cell lines by isomorellin. *Biol Pharm Bull* 2012; 35: 1914–25.
29 Banito A, Gil J. Induced pluripotent stem cells and senescence: learning the biology to improve the technology. *EMBO Rep* 2010; 11: 353–9.
30 Batheja N, Suriavinata A, Saxena R, Ionescu G, Schwartz M, Thung SN. Expression of p53 and PCNA in cholangiocarcinoma and primary sclerosing cholangitis. *Mod Pathol* 2000; 13: 1265–8.
31 Tokumoto N, Ikeda S, Ishizaki Y et al. Immunohistochemical and mutational analyses of Wnt signaling components and target genes in intrahepatic cholangiocarcinomas. *Int J Oncol* 2005; 27: 973–80.
32 Clevers H, Nusse R. Wnt/beta-catenin signaling and disease. *Cell* 2012; 149: 1192–205.
33 Shen DY, Zhang W, Zeng X, Liu CQ. Inhibition of Wnt/beta-catenin signaling downregulates P-glycoprotein and reverses multi-drug resistance of cholangiocarcinoma. *Cancer Sci* 2013; 104: 1303–8.
34 Xiao H, Ghosn C, Hinchman C et al. Adenomatous polyposis coli (APC)-independent regulation of beta-catenin degradation via a retinoid X receptor-mediated pathway. *J Biol Chem* 2003; 278: 29954–62.
35 Gu X, Ke S, Liu D et al. Role of NF-kappaB in regulation of PXR-mediated gene expression: a mechanism for the suppression of cytochrome P-450 3A4 by proinflammatory agents. *J Biol Chem* 2006; 281: 17882–9.