Study of the Effects of Cyclooxygenase-2 Inhibitor on the Promotion of Hepatic Tumorigenesis in Rats Fed a High Fat Diet

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Background/objective: Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide. The highest prevalence of hepatitis is an important risk factor contributing to development of HCCs. However, an increasing number of cases are associated metabolic disease and steatohepatitis. Inflammation associated with many liver disease, seems to be a necessary pre-requisite for successful tumor initiation. Mechanisms that link high fat diet and inflammation initial stage of HCC are not completely understood.

The present work was designed to investigate the effect of fat, through modulation of the insulin-like growth factors I and II (IGF-I and IGF-II), on the promotion of hepatocellular carcinoma, and the role of cyclooxygenase 2 (COX-2). **Methods:** two main groups of rats were used: control and HCC groups. The HCC group was further subdivide in to two subgroups, HCC fed with standard diet and HCC fed with high fat diet. The effects of celecoxib were also investigated in HCC fed with high fat diet. **Results:** We found that high fat diet was associated with significant increases in COX2 and interleukin 6 (IL6) with significant promotion of HCC progression. The significant increase in IGF could contribute partially to the observed effects of high fat diet. In addition, celecoxib was found to significantly reduce HCC progression. **Conclusions:** We conclude that COX2 could play central role in high prevalence of HCC observed with high fat diet. Several triggering factors such as IGF and IL6, together with the direct modulation of fat metabolism could open several novel preventive strategies of celecoxib treatment, and could be useful biomarkers for assessment of its pharmacological effects. (J CLIN EXP HEPATOL 2015;5:14-21)

The factors triggering hepatocellular carcinoma (HCC) are thought to be a complex combination involving genetic, infectious, metabolic, environmental and hormonal, which are all involved in complex way. Nutrition represent an important preventable risk factor in development of HCC, with high fat diet enhanced the development of hepatosteatois, ensuing liver damage and accelerating the appearance of liver tumors.^{1,2}

It was presumed that the Insulin-like growth factor (IGF) plays a key role in many neoplastic pathology, such as pediatric tumors, breast and colon cancer, as well as HCC. In addition, tumor cells with non-functional IGF receptors could induce their own proliferation by the synthesis of endogenous IGF molecules. This process of autocrine stimulation contributed in part to the autonomous and faster tumor growth.^{3,4}

Growing evidence indicates the inflammatory role of IGF in the initiation and progression of HCC. The important cancer-related inflammatory mediator that is up-regulated in most tumors is COX-2, which is documented to be an indicator of poor prognosis in many cancer types.^{5,6} In addition, Qiu et al⁷ reported that COX-2 may be involved in the early stages of hepatocarcinogenesis. Furthermore, it was reported that COX-2 expression in the tumor tissue was significantly correlated to the presence of inflammatory cells as macrophages and mast cells.^{8,9}

In the last few decades, extensive research effort continues to be devoted to the study of the anticancer mechanisms underlying diet modification.¹⁰ It was stated that besides taking steps to avoid hepatitis and cirrhosis, there is no reliable way to prevent liver cancer. However, the

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Abbreviations: AFP: alpha-fetoprotein; CCl4: carbon-tetrachloride; COX-2: cyclooxygenase 2; FAS: fatty acid synthase; FFA: free fatty acid; GH: growth hormone; H&E: Hematoxylin and Eosin stain; HCC: hepatocellular carcinoma; IκB: inhibitory protein; IGFBP-3: insulin-like growth factor-binding protein 3; IGF: IGF receptor; IGF-I and IGF-II: insulin-like growth factors I and II; IL6: interleukin 6; JNK1: c-Jun N-terminal kinase-1; MAPK: mitogen-activated protein kinase; NFκB: nuclear factor-κB; PI3k: phosphatidylinositolide 3-kinases; PAS: periodic acid Schiff stain; real time-PCR: real time-polymerase chain reaction; TAG: triacyl glycerol

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general cancer prevention guidelines include eating a high-fiber, low-fat diet, exercising, and avoiding alcohol and smoking.¹¹

The mechanism linking nutrients to the development of cancer is still not fully understood. We hypothesized that a functional link exists between COX-2, IGF and high fat levels in steatohepatitis associated HCC.

MATERIAL AND METHODS

Animals

After obtaining approval from the institutional Animal Care Committee, we used 48 male albino rats (body weight: 150–200 g, Laboratory Animal House Unit of Faculty of Medicine, Cairo University). The animals were housed in chip-bedded cages and, prior to experiments, acclimated for 1 week in the air conditioned institutional animal care unit. They were housed under 12 h light/dark cycles, with free access to water and standard rat chow.

General Protocol

Male rats were randomly assigned in to one of two groups: control group; represented placebo treated rats and hepatocellular carcinoma (HCC) group. HCC group was further divided in to three subgroups. Subgroup 1; HCC receiving standard rat chow (g/kg): Casein 200.0, Starch 615.0, Corn oil 80.0, Cellulose 50.0, Vitamin–Mineral Premix 50.0, DL-Methionine 3.0, Choline chloride 2.0, Chromium, (mg/kg) 0.066,¹² Subgroup 2; HCC animals received a high fat diet (g/kg): Casein 200.0, Starch 145.0, Sucrose—150.0, Cellulose 50.0, Beef tallow 400.0, Vitamin–Mineral Premix 50.0, DL-Methionine 3.0, Choline chloride 2.0, Chromium (mg/kg) 0.066.¹²

To investigate the role of COX2 in the progression of HCC promoted by high fat diet, subgroup 3 was added; in which HCC animals receiving a high fat diet were orally treated with celecoxib (Sigma aldrich, Cairo, Egypt) (3.6 mg twice/day oral for 6 weeks).

Hepatocarcinogenesis was induced chemically in rats by injecting a single intra-peritoneal dose of diethylnitrosamine (Sigma aldrich, Cairo, Egypt) at a dose of 200 mg/kg body weight, followed by weekly subcutaneous injections of Carbon-tetrachloride (CCl₄) (Sigma aldrich, Cairo, Egypt) at a dose of 3 mL/kg body weight for 6 weeks.¹³ At the planned time animals were sacrificed by cervical dislocation. The blood samples were collected for further measurement of Alpha-fetoprotein (AFP), Free fatty acid (FFA), triacyl glycerol (TAG), insulin like growth factor (IGF-I) and insulin like growth factor (IGF-II). In addition, liver samples were dissected for further histopathological assessment using Periodic acid Schiff stain (PAS), Masson's trichrome stain and Hematoxylin and Eosin (H&E) stain. Liver tissue gene expression of Cyclooxygenases (COX-2) and Interleukine-6 (IL-6) were also assessed.

Determination of Serum Alpha-Fetoprotein (AFP)

This was achieved by using rat Alpha-Fetoprotein (AFP) ELISA kit (Kamiya Biomedical company, Gateway drive, Seattle), which applies a technique called a quantitative sandwich immunoassay.

Determination of Serum Levels of Free Fatty Acids (FFA) and Triglycerides (TAG)

The serum FFA and TAG were measured by quantitative colorimetric technique. Biovision's Colorimetric and Fluorometric Assay Kit (BioVision, California, USA) provided a convenient, sensitive enzyme-based detection method, easily quantified by colorimetric (spectrophotometry at $\lambda = 570$ nm) in variety samples.

Determination of Insulin-like Growth Factor-I (IGF-I) and Insulin-like Growth Factor-II (IGF-II)

This was achieved by using rat IGF ELISA kit (Boster, Pleasanton, Canada), which was based on standard sandwich enzyme-linked immune-sorbent assay technology.

Real Time-Polymerase Chain Reaction (real time-PCR)

Real time-PCR (Pomega, Madison, WI, USA) was used to measure COX2 and IL-6 gene expression. The key equipment for real time-PCR is a specialized thermocycler with fluorescence detection modules, which is used to monitor and record the fluorescence in real time as amplification occurs. A typical workflow of real time-PCR for gene expression measurement involves RNA isolation, reverse transcription, real time-PCR assay development, real time-PCR experiment and data analysis.

Histo-pathological Examination

Liver samples were fixed in 10% paraformaldehyde and embedded in paraffin. Sections were obtained and stained with hematoxylin and eosin, Masson's trichrome stain and Periodic acid Schiff stain (PAS).

Statistical Methods

The results are given as means $\pm$ standard deviation (SD). Results were analyzed by using the software Prism 5 (GraphPad Software, La Jolla, CA, USA). Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test in normally distributed quantitative variables. Correlations were done to test for linear relations between quantitative variables by Pearson correlation. P-values less than or equal to 0.05 were considered as statistically significant.

Figure 1 (A, B): Role of celecoxib in hepatocellular (HCC) promotion effects associated with high fat diet. **A.** Development of steatohepatitis and pre-neoplastic lesions in livers male rats fed high fat chow. **(a)** Liver sections with normal architecture, normal hepatocytic appearance and normal portal tract appearance with neither inflammation nor fibrosis (H & E, $\times 100$). **(b)** Pronounced inflammatory infiltrate and bands of fibrotic tissue visualized by Masson's trichrome $\times 200$. **(c)** Glycogen empty hepatocytes with hydropic & fatty change (arrow) (PAS, $\times 100$). **(d)** Active cirrhosis with evidenced by inflammatory cells in septae (arrow) & inside nodules (star) (H & E, $\times 100$). **(e)** Dysplastic cells mostly small cell dysplasia (arrow) with dark nuclei, minimal dark cytoplasm and high nuclear/cytoplasmic ratio (H & E, $\times 200$). **(f)** Early preneoplastic hepatocyte changes most properly of small cell dysplasia in a cirrhotic nodule (H & E, $\times 400$). **(g)** Areas of PAS negative empty cells indicating degenerative changes in hepatocytes with marked increase in vascularity and congestion (arrow) (PAS, $\times 100$). **B.** Celecoxib treatment significantly reduced the serum level of Alpha-fetoprotein (AFP). \$ vs control; * vs HCC with normal diet regimen; # vs HCC with high fat diet regimen.

RESULTS

Effects of High Fat Diet on the Development of Liver Dysplasia and Hepatocellular Carcinoma

In spite that gross examination revealed no differences among all groups, microscopic examination of liver sec-

tions demonstrated various stages of development of HCC including fatty changes, inflammation, fibrosis, and cirrhosis and dysplastic changes were observed more obviously in HCC rats fed with normal diet. More micro and macrovesicular fatty changes, focal cell replacement by inflammatory cell, focal necrosis, dysplastic cells and marked

Figure 2 (A–D): Effects of celecoxib on the characterised changes in the serum levels of growth factors [insulin like growth factor-I (IGF-I) (A) and Insulin like growth factor-II (IGF-II) (B)], free fatty acid (FFA) (C) and triacyl glycerol (TAG) (D) associated with high fat diet in hepatocellular carcinoma (HCC). \$ vs control; * vs HCC with normal diet regimen; # vs HCC with high fat diet regimen.

congestion of central vein with degenerative changes in hepatocytes obtained from HCC rats fed with high fat diet (Figure 1a).

In our study, high fat diet was also associated with significant increases ($P \leq 0.05$) in the level of AFP (ng/dl) [2.9 ± 0.7] in comparison to both control [0.4 ± 0.1] and HCC rats receiving normal diet regimen [1.9 ± 0.4] (Figure 1b).

Previous described oncogenic promotion effects of high fat diet was associated with significant increase ($P \leq 0.05$) in serum levels of IGF1 (Pg/ml) [100.7 ± 11.3], IGF2 (Pg/ml) [125.2 ± 21.9], FFA (mg/dl) [1.4 ± 0.6] and TAG (mg/dl) [93.2 ± 8.9] in comparison to both control IGF1 (Pg/ml) [30.3 ± 3.3], IGF2 (Pg/ml) [35.49 ± 4.789], FFA (mg/dl) [0.57 ± 0.18] and TAG (mg/dl) [58 ± 6.8] and HCC rats fed with normal diet regimen IGF1 (Pg/ml) [71.68 ± 17.27], IGF2 (Pg/ml) [76.28 ± 15.78], FFA (mg/dl) [0.56 ± 0.14] and TAG (mg/dl) [55.38 ± 6.5] (Figure 2(A–D)).

In addition, we observed significant increase ($P \leq 0.05$) in the inflammatory markers gene expression [COX2: 0.2 ± 0.09 and IL6: 10.1 ± 1.9] in HCC fed with high fat diet in comparison to control group [COX2: 0.01 ± 0.01 and IL6: 1.16 ± 0.21] and HCC rats fed with normal diet regimen [COX2: 0.11 ± 0.03 and IL6: 4.34 ± 1.7] (Figure 3(A, B)).

Effects of Celecoxib on the Development of Liver Dysplasia and Hepatocellular Carcinoma with High Fat Diet

Microscopic examination of liver sections demonstrated the same fatty changes with less inflammatory, vascular, dysplastic and degenerative changes previously observed in untreated HCC rats with high fat diet (Figure 1a).

Furthermore, inhibition of COX2 by Celecoxib induced significant decreases ($P \leq 0.05$) in the level of AFP (ng/dl)

Figure 3 Anti-inflammatory effects of celecoxib on the inflammatory changes associated with high fat diet hepatocarcinogenic effects. Celecoxib was associated with significant reduction of cyclooxygenase 2 (COX2) (A) and interleukin 6 (IL6) (B) gene expression significantly induced in hepatocellular carcinoma (HCC) fed with high fat diet. \$ vs control; * vs HCC with normal diet regimen; # vs HCC with high fat diet regimen.

[1.8 ± 0.3] in comparison to untreated HCC rats with high fat diet (Figure 1b).

In addition, celecoxib treatment was associated with significant reduction ($P \leq 0.05$) in serum levels of both IGF2 (Pg/ml) [103.3 ± 6.9] and FFA (mg/dl) [1.09 ± 0.2]. However, no significant differences ($P > 0.05$) were observed in measured serum levels of IGF1 and TAG (Figure 2(A–D)).

Anti-inflammatory effects of celecoxib were observed also in HCC fed with high fat diet through significant reduction in gene expression of both COX2 [0.1 ± 0.04] and IL6 [6.9 ± 0.8] (Figure 3(A, B)).

Effects of Fat, Growth Factors and IL6 on Cyclooxygenase 2 Gene Expression

Strong positive correlations ($P \leq 0.05$) were observed between IGF I, IGFII, FFA, TAG, IL6 and COX2 gene expression [$r = 0.68, 0.72, 0.45, 0.51, 0.73$ respectively] (Figure 4 (A–E)).

DISCUSSION

We found that high fat diet-through modulation of IGF I and IGFII- is one of the determinant factors that contribute to progress of HCC. Celecoxib could play an important role in the observed steatohepatitis and HCC, an effect that could be contributed to its anti-inflammatory property, modulation of IGF secretion and modulation of fat metabolism (Figure 5).

Recently the link has been investigated between chronic inflammation and several cancers.¹⁴ While Up-regulation of COX-2 in solid malignancies including colon, prostate,

breast, pancreas, non-small-cell lung, bladder, endometrium and skin basal and squamous cell has been previously reported,^{15–18} few studies investigated the role of COX in HCC. In addition, biomarkers able to select the patients who are most likely to benefit from celecoxib, and to monitor its pharmacological effects are issues that need further investigations.

High fat diet induces development of HCC through a series of steps: lipid accumulation in the liver, peroxidation of these lipids and induction of inflammation (hepatitis) and ultimately this continuous process.^{19,20} Consumption of a high-fat diet has been reported to activate nuclear factor- κ B (NF κ B) via its release from binding to the inhibitory protein (I κ B) in the cytoplasm, an effect that increase cytokines release and trigger cell proliferation.²¹

We found that high fat diet promotes HCC, chemically induced by common two stage model in which the initiation by a genotoxic compound (N-nitrosodiethylamine (DEN)) is followed by a promotion phase induced by carbon-tetrachloride (CCl₄), that mimic the injury-fibrosis-malignancy cycle.²² The observed effect of high fat diet is associated with up-regulation of Cyclooxygenase 2 (COX2) gene expression, an enzyme that could contribute to the observed hepatocellular inflammatory and dysplastic changes associated with steatohepatitis.

In our study, high fat diet was associated with significant increase in the serum levels of both IGF1 and IGF2. Clinical studies reported the oncogenic roles of IGFs, through their binding to IGF receptor (IGFR) and activation of Mitogen-activated protein kinase (MAPK), an effect that contribute to COX-2 mRNA expression.^{23–28}

Figure 4 (A–E): Effects of growth factors, fat and interleukin 6 on cyclooxygenase 2 (COX2) gene expression. Strong positive correlation between insulin growth factor I (IGF I) (A), IGF II (B), free fatty acid (FFA) (C), triacyl glycerol (TAG) (D) and cyclooxygenase 2 (COX2) gene expression. And strong negative correlation between interleukin 6 gene expression (E) and COX2 gene expression.

In addition to major central role of growth hormone (GH) in regulation of IGF secretion, circulating IGF concentration is also affected by other factors. Recently, nutrition was found to be an important determinant factor. Although protein-calorie malnutrition was reported to decrease IGF-I levels,²⁹ few published studies have related high fat diet to IGF-I and IGF 2 levels and have reported that specific fatty

acids may enhance cancer invasion and metastatic potential.^{30–32} High fat diet, through hyperinsulinemia and insulin resistance could directly influence the hepatic production of IGF, especially IGF2 that is produced mainly by malignant rather than normal cells.^{33–35}

While Pold et al³⁶ found that COX-2 is modulator of the IGF axis in lung cancer, and that over expression of COX2

Figure 5 Hepatocellular carcinoma (HCC) promoting effects of high fat diet. Solid arrows represent trigger factors for HCC different events, interrupted arrows represent the effects of cyclooxygenase 2 (COX2) on the fat level, interleukin 6 and insulin growth factor receptor (IGFR) axis.

with tumor was associated with high systemic levels of IGF-1 and the insulin-like growth factor-binding protein 3 (IGFBP-3), we found that inhibition of COX2 significantly decreased the serum level of IGF2 rather than IGF1. This difference could be due to differences in experimental circumstances. Furthermore, the significant effect of celecoxib on the serum level of IGF2, main growth factor associated with HCC, rather than IGF1 seems to further support the anti-oncogenic effects of celecoxib, with reduction of malignant cell IGF production ability.^{27,37,38}

The observed anti-oncogenic effect of celecoxib could partially be due to its effects on lipid metabolism.³⁹ Controversial to Lu et al,⁴⁰ who reported that Celecoxib reduced TAG levels in mammalian tumor promoted by high fat diet and this could contribute to non COX2 effects on fat metabolism, we found that Celecoxib induced differential effects on the serum levels of FFA and TAG, with significant decrease in FFA levels and no significant effect on TAG, after treatment in HCC group that was fed with high fat diet. This could be due to differences in experimental protocol and type of diet regimen we used. Celecoxib induces modulation in multiple gene expression resulting in alterations in cellular lipid metabolism and glutathione metabolism. Lu et al⁴¹ observed that fatty acid synthase (FAS) was significantly down-regulated by celecoxib treatment through down-regulation of c-Jun N-terminal kinase-1 (JNK1) in rat hepatocytes, an effect that could explain the low FFA associated with celecoxib treatment.

Our study has limitations: First; we measured only serum rather than liver expression of IGF. However Kern et al⁴² previously reported that IGF is over-expressed in 30–40% of HCCs. Second; we only investigated the effects of high fat diet and celecoxib on the IL6 rather than other cytokines. However, it was suggested that IL6 increased expression has posed major barriers limiting the efficacy of chemotherapy and hence cancer treatment.^{5,43} Both Johnson et al⁴⁴ and Giannitrapani et al⁴⁵ reported that IL-6 levels were significantly higher in HCC patients when compared to cirrhotic patients who do not have cancer. Third; one celecoxib dose and one time point were

investigated. However, our aim was to investigate the effects of celecoxib on the early post-initiation inflammatory events of HCC and thus prevention of further malignant progression.

CONCLUSION

Investigation the role of COX among down-stream pathways of the various initiating factors are important for understanding mechanisms leading to HCCs associated with high fat diet, identifying diagnostic markers (such as IGF and IL6) and developing prophylactic measures and effective treatments (such as celecoxib).

CONFLICTS OF INTEREST

All authors have none to declare.

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REFERENCES

- Shaker MK, Abdella HM, Khalifa MO, El Dorry AK. Epidemiological characteristics of hepatocellular carcinoma in Egypt: a retrospective analysis of 1313 cases. *Liver Int.* 2013;33:1601–1606.
- Yang S, Lin HZ, Hwang J, Chacko VP, Diehl AM. Hepatic hyperplasia in noncirrhotic fatty livers: is obesity-related hepatic steatosis a premalignant condition? *Cancer Res.* 2001;61:5016–5023.
- Pavelic J, Matijevic T, Knezevic J. Biological & physiological aspects of action of insulin-like growth factor peptide family. *Indian J Med Res.* 2007;125:511–522.
- Knowlden JM, Jones HE, Barrow D, Gee JM, Nicholson RI, Hutcheson IR. Insulin receptor substrate-1 involvement in epidermal growth factor receptor and insulin-like growth factor receptor signalling: implication for Gefitinib ('Iressa') response and resistance. *Breast Cancer Res Treat.* 2008;111:79–91.
- Hursting SD, Dunlap SM, Ford NA, Hursting MJ, Lashinger LM. Calorie restriction and cancer prevention: a mechanistic perspective. *Cancer Metab.* 2013;1:10.
- Koki A, Khan NK, Woerner BM, et al. Cyclooxygenase-2 in human pathological disease. *Adv Exp Med Biol.* 2002;507:177–184.
- Qiu DK, Ma X, Peng YS, Chen XY. Significance of cyclooxygenase-2 expression in human primary hepatocellular carcinoma. *World J Gastroenterol.* 2002;8:815–817.
- Cervello M, Montalto G. Cyclooxygenases in hepatocellular carcinoma. *World J Gastroenterol.* 2006;12:5113–5121.
- Cervello M, Foderà D, Florena AM, et al. Correlation between expression of cyclooxygenase-2 and the presence of inflammatory cells in human primary hepatocellular carcinoma: possible role in tumor promotion and angiogenesis. *World J Gastroenterol.* 2005;11:4638–4643.
- Fontana L, Weiss EP, Villareal DT, Klein S, Holloszy JO. Long-term effects of calorie or protein restriction on serum IGF-1 and IGFBP-3 concentration in humans. *Aging Cell.* 2008;7:681–687.
- Gantos-O'Brien E. A patient's perspective on the multidisciplinary liver/pancreas tumor clinic: an all-in-one resort. *J Oncol Pract.* 2010;6:292–294.
- Tuzcu M, Sahin N, Orhan C, et al. Impact of chromium histidinate on high fat diet induced obesity in rats. *Nutr Metab (Lond).* 2011;8:28.

13. Abdel aziz MT, El Asmar MF, Atta HM, et al. Efficacy of mesenchymal stem cells in suppression of hepatocarcinogenesis in rats: possible role of Wnt signaling. *J Exp Clin Cancer Res*. 2011;30:49.
14. Hill-Baskin AE, Markiewski MM, Buchner DA, et al. Diet-induced hepatocellular carcinoma in genetically predisposed mice. *Hum Mol Genet*. 2009;18:2975–2988.
15. Jou J, Choi SS, Diehl AM. Mechanisms of disease progression in nonalcoholic fatty liver disease. *Semin Liver Dis*. 2008;28:370–379.
16. Zhang X, Dong F, Ren J, Driscoll MJ, Culver B. High dietary fat induces NADPH oxidase-associated oxidative stress and inflammation in rat cerebral cortex. *Exp Neurol*. 2005;191:318–325.
17. Sobolewski C, Cerella C, Dicato M, Ghibelli L, Diederich M. The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies. *Int J Cell Biol*. 2010;215:158.
18. Lim SC, Lee TB, Choi CH, Ryu SY, Kim KJ, Min YD. Expression of cyclooxygenase-2 and its relationship to p53 accumulation in colorectal cancers. *Yonsei Med J*. 2007;48:495–501.
19. Anai S, Tanaka M, Shiverick KT, et al. Increased expression of cyclooxygenase-2 correlates with resistance to radiation in human prostate adenocarcinoma cells. *J Urol*. 2007;177:1913–1917.
20. Parrett M, Harris R, Joarder F, Ross M, Clausen K, Robertson F. Cyclooxygenase-2 gene expression in human breast cancer. *Int J Oncol*. 1997;10:503–507.
21. Mazhar D, Gillmore R, Waxman J. COX and cancer. *QJM*. 2005;98:711–718.
22. Chang YS, Kong G, Sun S, et al. Clinical significance of insulin-like growth factor-binding protein-3 expression in stage I non-small cell lung cancer. *Clin Cancer Res*. 2002;8:3796–3802.
23. Kozaki K, Koshikawa K, Tatematsu Y, et al. Multi-faceted analyses of a highly metastatic human lung cancer cell line NCI-H460-LNM35 suggest mimicry of inflammatory cells in metastasis. *Oncogene*. 2001;20:4228–4234.
24. Yasumaru M, Tsuji S, Tsujii M, et al. Inhibition of angiotensin II activity enhanced the antitumor effect of cyclooxygenase-2 inhibitors via insulin-like growth factor I receptor pathway. *Cancer Res*. 2003;63:6726–6734.
25. Guan Z, Buckman SY, Baier LD, Morrison AR. IGF-I and insulin amplify IL-1 beta-induced nitric oxide and prostaglandin biosynthesis. *Am J Physiol*. 1998;274(4 Pt 2):F673–F679.
26. Wu J, Zhu AX. Targeting insulin-like growth factor axis in hepatocellular carcinoma. *J Hematol Oncol*. 2014;4:30.
27. LeRoith D, Roberts CT. The insulin-like growth factor system and cancer. *Cancer Lett*. 2003;195:127–137.
28. Thissen JP, Ketelslegers JM, Underwood LE. Nutritional regulation of the insulin-like growth factors. *Endocr Rev*. 1994;15:80–101.
29. Ma J, Stampfer M, Pollak M. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. *J Natl Cancer Inst*. 1999;91:620–625.
30. Holmes MD, Pollak MN, Willett WC, Hankinson SE. Dietary correlates of plasma insulin-like growth factor I and insulin-like growth factor binding protein 3 concentrations. *Cancer Epidemiol Biomarkers Prev*. 2002;11:852–861.
31. Willett WC. Specific fatty acids and risks of breast and prostate cancer: dietary intake. *Am J Clin Nutr*. 1997;66(6 suppl 1):1557S–1563S.
32. Pollak M. Targeting insulin and insulin-like growth factor signalling in oncology. *Curr Opin Pharmacol*. 2008;8:384–392.
33. Gallagher EJ, Fierz Y, Ferguson RD, LeRoith D. The pathway from diabetes and obesity to cancer, on the route to targeted therapy. *Endocr Pract*. 2010;16:864–873.
34. D'Ercole AJ, Stiles AD, Underwood LE. Tissue concentrations of somatomedin C: further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms of action. *Proc Natl Acad Sci USA*. 1984;81:935–939.
35. Pold M, Krysan K, Pöld A, et al. Cyclooxygenase-2 modulates the insulin-like growth factor axis in non-small-cell lung cancer. *Cancer Res*. 2004;64:6549–6555.
36. Zhou Q, Mao YQ, Jiang WD, et al. Development of IGF signaling antibody arrays for the identification of hepatocellular carcinoma biomarkers. *PLoS One*. 2012;7:e46851.
37. Mukherjee B, Ghosh S, Das T, Doloi M. Characterization of insulin-like-growth factor II (IGF II) mRNA positive hepatic altered foci and IGF II expression in hepatocellular carcinoma during diethylnitrosamine-induced hepatocarcinogenesis in rats. *J Carcinog*. 2005;4:12.
38. Heindryckx F, Colle I, Van Vlierberghe H. Experimental mouse models for hepatocellular carcinoma research. *Int J Exp Pathol*. 2009;90:367–386.
39. Kannen V, Zanette DL, Fernandes CR, et al. High-fat diet causes an imbalance in the colonic serotonergic system promoting adipose tissue enlargement and dysplasia in rats. *Toxicol Lett*. 2012;213:135–141.
40. Lu S, Zhang X, Badawi AF, El-Sohehy A, Archer MC. Cyclooxygenase-2 inhibitor celecoxib inhibits promotion of mammary tumorigenesis in rats fed a high fat diet rich in n-6 polyunsaturated fatty acids. *Cancer Lett*. 2002;184:7–12.
41. Lu S, Archer MC. Celecoxib decreases fatty acid synthase expression via down-regulation of c-Jun N-terminal kinase-1. *Exp Biol Med (Maywood)*. 2007;232:643–653.
42. Kern MA, Schöneweiß MM, Sahi D. Cyclooxygenase-2 inhibitors suppress the growth of human hepatocellular carcinoma implants in nude mice. *Carcinogenesis*. 2004;25:1193–1199.
43. Duan Z, Lamendola DE, Penson RT, Kronish KM, Seiden MV. Overexpression of IL-6 but not IL-8 increases paclitaxel resistance of U-2OS human osteosarcoma cells. *Cytokine*. 2002;17:234–242.
44. Johnson C, Han Y, Hughart N, McCarra J, Alpini G, Meng F. Interleukin-6 and its receptor, key players in hepatobiliary inflammation and cancer. *Transl Gastrointest Cancer*. 2012;1:58–70.
45. Giannitrapani L, Soresi M, Giacalone A, et al. IL-6-174G/C polymorphism and IL-6 serum levels in patients with liver cirrhosis and hepatocellular carcinoma. *OMICS*. 2011;15:183–186.