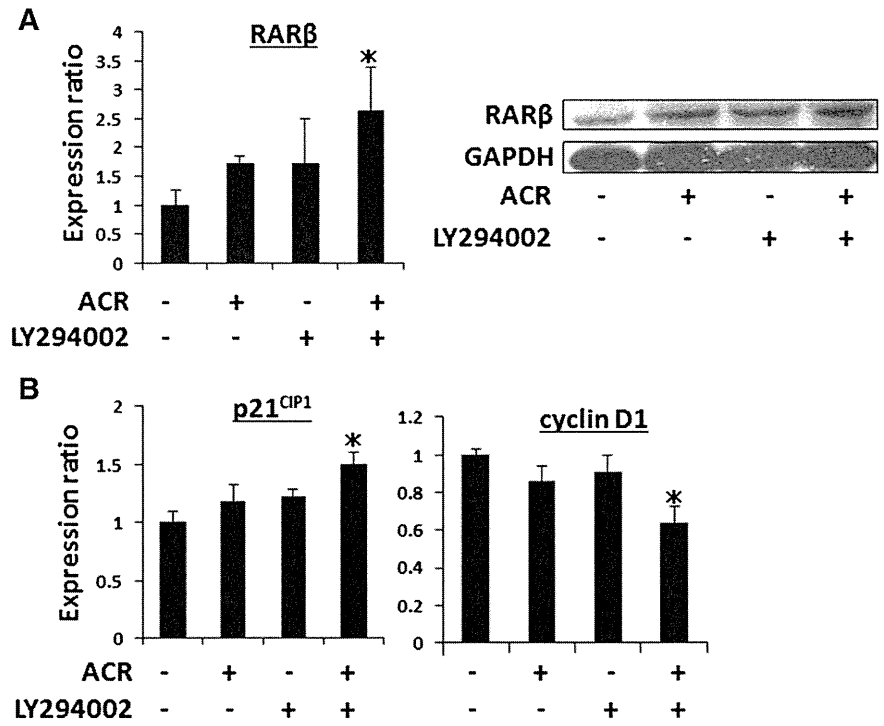

Figure 6 Effects of the combination of ACR and LY294002 on the cellular expression levels of RARβ, $p21^{CIP1}$, and cyclin D1 in HLF cells. **(A)** The expression levels of RARβ mRNA (left panel) and protein (right panel) were examined by quantitative real-time RT-PCR analysis and western blot analysis, respectively, using cells treated with the test drugs for 24 hours. **(B)** Quantitative real-time RT-PCR analysis to examine the expression levels of $p21^{CIP1}$ and cyclin D1 mRNAs were performed using cells treated with the test drugs for 24 hours. The expression level of each mRNA was normalized to the level of β-actin mRNA. Values represent the means ± SD of triplicate analyses. * $P < 0.05$.

findings suggest that the combination of ACR and LY294002 cooperatively inhibit the phosphorylation of RXRα through dephosphorylation of ERK and Akt, which leads to the synergistic inhibition of growth and the induction of apoptosis in HCC cells. The results of the present research, together with those of previous studies [17,25,28-30], suggest that dephosphorylation of RXRα might be a key mechanism for ACR-based combination chemoprevention in HCC cells.

Phosphorylated RXRα loses its ability to form heterodimers with RARβ and this is associated with resistance to retinoids [7]. Therefore, restoration of the function of RXRα by inhibiting its phosphorylation is critical to regulate the expression of retinoid target genes [4-9]. In comparison to treatment with ACR alone or LY294002 alone, combined treatment with these agents significantly increased the transcriptional activity of the RXRE reporter in the present study. This combination also significantly altered the expression levels of ACR target genes, such as RARβ, $p21^{CIP1}$, and cyclin D1 mRNA [13,25,27,34]. Particularly, the induction of RARβ by the combination of ACR and LY294002 might play a crucial role in inhibiting the growth of HCC cells because RARβ, which is a receptor for ACR [36], can exert tumor-suppressive effects in cancer cells and thus be considered as a tumor suppressor gene [37].

In this study, the phosphorylation of Akt is inhibited by ACR alone in HLF cells. This finding seems to be of interest because Akt phosphorylation plays a critical role in cell survival, prevention of apoptosis, and progression of cell cycle in various types of tumors, including HCC [21,22]. The precise mechanism by which ACR inhibits the phosphorylation of Akt protein has not been determined. However, we assume that the dephosphorylation of this protein by ACR might be explained by, at least in part, its ability to inhibit growth factor-dependent RTK activity, because Akt is potently phosphorylated by the activation of RTKs [8,9,14,15,18-20]. For instance, ACR inhibits the growth of HCC cells and prevents chemically induced liver tumorigenesis by targeting the transforming growth factor-α/epidermal growth factor receptor (EGFR) axis, which belongs to RTKs [14,15]. Moreover, a recent study showed that retinol inhibited PI3K activity by decreasing the interaction between PI3K and phosphatidylinositol and this was associated with suppression of cell growth in colon cancer cells [38]. These studies suggest that the PI3K/Akt signaling pathway might be a critical target for retinoids to exert their anti-cancer and chemopreventive properties.

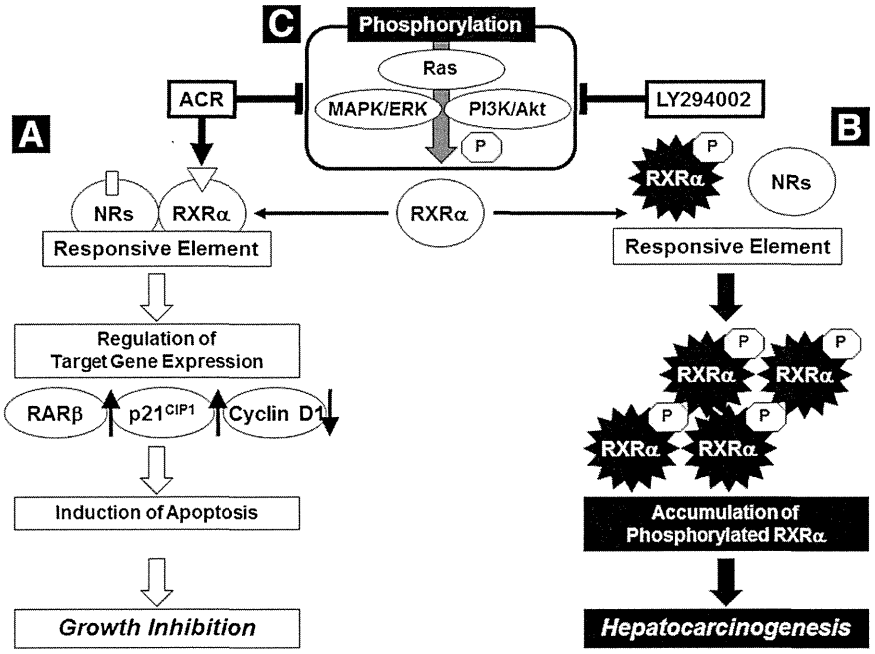

Figure 7 A hypothetical schematic representation of the effects of the combination of ACR and LY294002 on growth inhibition in HCC cells. When ACR binds to and activates RXRα, it forms homo- and/or heterodimers with other nuclear receptors (NRs), including RARs. This results in the activation of the transcriptional activity of the responsive element, thus controlling the expression of the target genes, such as RARβ, $p21^{CIP1}$, and cyclin D1, which induce apoptosis and inhibit the growth of HCC cells **(A)**. In HCC cells, the MAPK/ERK and PI3K/Akt pathways, both of which are located downstream of Ras, are highly activated and phosphorylate the RXRα protein. The accumulation of phosphorylated RXRα protein, which impairs dimer formation and the subsequent transactivation functions of this receptor, cause a deviation from normal cell proliferation and differentiation, thereby playing a critical role in liver carcinogenesis **(B)**. ACR and LY294002 inhibit RXRα phosphorylation by inhibiting ERK and Akt phosphorylation, resulting in restoration of receptor function and activation of the transcriptional activity of the responsive element **(C)**. For additional details, see the Discussion section.

In the current study, the combination of ACR and LY294002 significantly inhibited the growth of HLF, Huh7, and Hep3B HCC cells, whereas the growth of HepG2 cells, the other HCC cell line, was not suppressed by this combination. This might be associated with the phosphorylation status of ERK and Akt proteins because the expression levels of p-ERK and p-Akt proteins were increased in HLF, Huh7, and Hep3B cells compared with HepG2 cells [29]. These results, on the other hand, suggest that HCC cells that overexpress p-ERK and p-Akt proteins might be more sensitive targets for combination therapy using ACR and PI3K inhibitors.

Finally, it should be emphasized that combination therapy and prevention are advantageous because, in addition to providing the potential for synergistic effects, they may reduce the opportunity for the development of drug resistance by cancer cells. Several preclinical studies have shown that cancer cells harboring activated Ras mutations appear to be resistant to treatment with PI3K inhibitor alone [23,39]. However, the use of a combination of the PI3K/Akt inhibitor and a MAPK inhibitor significantly exerted anti-cancer effects in *Kars* G12D-driven or EGFR-mutant lung tumors [23,24]. These studies suggest that effective treatment with PI3K inhibitors require concomitant therapies that target RTK/Ras/MAPK signaling and, therefore, ACR, which can inhibit this signaling pathway [8,9,14,15,40], might be a preferable partner for PI3K inhibitors.

In conclusion, the present study indicates that the combination of ACR and LY294002, which can inhibit the phosphorylation of RXRα, causes a synergistic induction of apoptosis and inhibition of cell growth in human HCC cells. The results of our study suggest that this combination might hold promise as a clinical modality for the prevention and treatment of HCC, due to their synergistic effects. In particular, our finding that the combination regimen using 1 μM ACR plus 5 μM LY294002 synergistically inhibits the growth of HCC cells seems to be clinically relevant because this concentration (1 μM) is approximately the same as the plasma concentration of ACR (which ranged from 1 to 5 μM) in a clinical trial that demonstrated the chemopreventive effects of this agent in the recurrence of secondary HCC [10,11].

Abbreviations
ACR: Acyclic retinoid; CI: Combination index; DMEM: Dulbecco's modified eagle medium; EGFR: Epidermal growth factor receptor; ERK: Extracellular signal-regulated kinase; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; HCC: Hepatocellular carcinoma; IFN: Interferon; MAPK: Mitogen-activated protein kinase; PI3K: Phosphatidylinositol 3-kinase; RAR: Retinoic acid receptor; RTK: Receptor tyrosine kinase; RT-PCR: Reverse transcription PCR; RXR: Retinoid X receptor; RXRE: Retinoid X receptor response element; TUNEL: Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
AB, MS, and TO conceived of the study, participated in its design, and drafted the manuscript. AB, MS, TO, YS, MK, and TK performed in vitro experiment. DT performed statistical analysis. HT and HM helped to draft the manuscript. All authors read and approved the final manuscript.

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References

1. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM: **The nuclear receptor superfamily: the second decade.** *Cell* 1995, **83**:835–839.
2. Chambon P: **A decade of molecular biology of retinoic acid receptors.** *FASEB J* 1996, **10**:940–954.
3. Altucci L, Leibowitz MD, Ogilvie KM, de Lera AR, Gronemeyer H: **RAR and RXR modulation in cancer and metabolic disease.** *Nat Rev Drug Discov* 2007, **6**:793–810.
4. Matsushima-Nishiwaki R, Okuno M, Takano Y, Kojima S, Friedman SL, Moriwaki H: **Molecular mechanism for growth suppression of human hepatocellular carcinoma cells by acyclic retinoid.** *Carcinogenesis* 2003, **24**:1353–1359.
5. Matsushima-Nishiwaki R, Okuno M, Adachi S, Sano T, Akita K, Moriwaki H, Friedman SL, Kojima S: **Phosphorylation of retinoid X receptor alpha at serine 260 impairs its metabolism and function in human hepatocellular carcinoma.** *Cancer Res* 2001, **61**:7675–7682.
6. Adachi S, Okuno M, Matsushima-Nishiwaki R, Takano Y, Kojima S, Friedman SL, Moriwaki H, Okano Y: **Phosphorylation of retinoid X receptor suppresses its ubiquitination in human hepatocellular carcinoma.** *Hepatology* 2002, **35**:332–340.
7. Yoshimura K, Muto Y, Shimizu M, Matsushima-Nishiwaki R, Okuno M, Takano Y, Tsurumi H, Kojima S, Okano Y, Moriwaki H: **Phosphorylated retinoid X receptor alpha loses its heterodimeric activity with retinoic acid receptor beta.** *Cancer Sci* 2007, **98**:1868–1874.
8. Shimizu M, Takai K, Moriwaki H: **Strategy and mechanism for the prevention of hepatocellular carcinoma: phosphorylated retinoid X receptor alpha is a critical target for hepatocellular carcinoma chemoprevention.** *Cancer Sci* 2009, **100**:369–374.
9. Shimizu M, Sakai H, Moriwaki H: **Chemoprevention of hepatocellular carcinoma by acyclic retinoid.** *Front Biosci* 2011, **16**:759–769.
10. Muto Y, Moriwaki H, Ninomiya M, Adachi S, Saito A, Takasaki KT, Tanaka T, Tsurumi K, Okuno M, Tomita E, Nakamura T, Kojima T: **Prevention of second primary tumors by an acyclic retinoid, polyprenoic acid, in patients with hepatocellular carcinoma. Hepatoma prevention study group.** *N Engl J Med* 1996, **334**:1561–1567.
11. Muto Y, Moriwaki H, Saito A: **Prevention of second primary tumors by an acyclic retinoid in patients with hepatocellular carcinoma.** *N Engl J Med* 1999, **340**:1046–1047.
12. Suzui M, Masuda M, Lim JT, Albanese C, Pestell RG, Weinstein IB: **Growth inhibition of human hepatoma cells by acyclic retinoid is associated with induction of p21(CIP1) and inhibition of expression of cyclin D1.** *Cancer Res* 2002, **62**:3997–4006.
13. Suzui M, Shimizu M, Masuda M, Lim JT, Yoshimi N, Weinstein IB: **Acyclic retinoid activates retinoic acid receptor beta and induces transcriptional activation of p21(CIP1) in HepG2 human hepatoma cells.** *Mol Cancer Ther* 2004, **3**:309–316.
14. Nakamura N, Shidoji Y, Moriwaki H, Muto Y: **Apoptosis in human hepatoma cell line induced by 4,5-didehydro geranylgeranoic acid (acyclic retinoid) via down-regulation of transforming growth factor-alpha.** *Biochem Biophys Res Commun* 1996, **219**:100–104.
15. Kagawa M, Sano T, Ishibashi N, Hashimoto M, Okuno M, Moriwaki H, Suzuki R, Kohno H, Tanaka T: **An acyclic retinoid, NIK-333, inhibits N-diethylnitrosamine-induced rat hepatocarcinogenesis through suppression of TGF-alpha expression and cell proliferation.** *Carcinogenesis* 2004, **25**:979–985.
16. Shimizu M, Sakai H, Shirakami Y, Iwasa J, Yasuda Y, Kubota M, Takai K, Tsurumi H, Tanaka T, Moriwaki H: **Acyclic retinoid inhibits diethylnitrosamine-induced liver tumorigenesis in obese and diabetic C57BLKS/J- + (db)/+Lepr(db) mice.** *Cancer Prev Res* 2011, **4**:128–136.
17. Shimizu M, Shirakami Y, Sakai H, Iwasa J, Shiraki M, Takai K, Naiki T, Moriwaki H: **Combination of acyclic retinoid with branched-chain amino acids inhibits xenograft growth of human hepatoma cells in nude mice.** *Hepatol Res* 2012, **42**:1241–1247.
18. Engelman JA: **Targeting PI3K signalling in cancer: opportunities, challenges and limitations.** *Nat Rev Cancer* 2009, **9**:550–562.
19. Courtney KD, Corcoran RB, Engelman JA: **The PI3K pathway as drug target in human cancer.** *J Clin Oncol* 2010, **28**:1075–1083.
20. Vivanco I, Sawyers CL: **The phosphatidylinositol 3-Kinase AKT pathway in human cancer.** *Nat Rev Cancer* 2002, **2**:489–501.
21. Zhou Q, Lui VW, Yeo W: **Targeting the PI3K/Akt/mTOR pathway in hepatocellular carcinoma.** *Future Oncol* 2011, **7**:1149–1167.
22. Llovet JM, Bruix J: **Molecular targeted therapies in hepatocellular carcinoma.** *Hepatology* 2008, **48**:1312–1327.
23. Engelman JA, Chen L, Tan X, Crosby K, Guimaraes AR, Upadhyay R, Maira M, McNamara K, Perera SA, Song Y, Chirieac LR, Kaur R, Lightbown A, Simendinger J, Li T, Padera RF, Garcia-Echeverria C, Weissleder R, Mahmood U, Cantley LC, Wong KK: **Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers.** *Nat Med* 2008, **14**:1351–1356.
24. Faber AC, Li D, Song Y, Liang MC, Yeap BY, Bronson RT, Lifshits E, Chen Z, Maira SM, Garcia-Echeverria C, Wong KK, Engelman JA: **Differential induction of apoptosis in HER2 and EGFR addicted cancers following PI3K inhibition.** *Proc Natl Acad Sci U S A* 2009, **106**:19503–19508.
25. Tatebe H, Shimizu M, Shirakami Y, Sakai H, Yasuda Y, Tsurumi H, Moriwaki H: **Acyclic retinoid synergises with valproic acid to inhibit growth in human hepatocellular carcinoma cells.** *Cancer Lett* 2009, **285**:210–217.
26. Obora A, Shiratori Y, Okuno M, Adachi S, Takano Y, Matsushima-Nishiwaki R, Yasuda I, Yamada Y, Akita K, Sano T, Shimada J, Kojima S, Okano Y, Friedman SL, Moriwaki H: **Synergistic induction of apoptosis by acyclic retinoid and interferon-beta in human hepatocellular carcinoma cells.** *Hepatology* 2002, **36**:1115–1124.
27. Shimizu M, Suzui M, Deguchi A, Lim JT, Xiao D, Hayes JH, Papadopoulos KP, Weinstein IB: **Synergistic effects of acyclic retinoid and OSI-461 on growth inhibition and gene expression in human hepatoma cells.** *Clin Cancer Res* 2004, **10**:6710–6721.
28. Kanamori T, Shimizu M, Okuno M, Matsushima-Nishiwaki R, Tsurumi H, Kojima S, Moriwaki H: **Synergistic growth inhibition by acyclic retinoid and vitamin K2 in human hepatocellular carcinoma cells.** *Cancer Sci* 2007, **98**:431–437.
29. Tatebe H, Shimizu M, Shirakami Y, Tsurumi H, Moriwaki H: **Synergistic growth inhibition by 9-cis-retinoic acid plus trastuzumab in human hepatocellular carcinoma cells.** *Clin Cancer Res* 2008, **14**:2806–2812.
30. Ohno T, Shirakami Y, Shimizu M, Kubota M, Sakai H, Yasuda Y, Kochi T, Tsurumi H, Moriwaki H: **Synergistic growth inhibition of human hepatocellular carcinoma cells by acyclic retinoid and GW4064, a farnesoid X receptor ligand.** *Cancer Lett* 2012, **323**:215–222.
31. Zhao L, Wientjes MG, Au JL: **Evaluation of combination chemotherapy: integration of nonlinear regression, curve shift, isobologram, and combination index analyses.** *Clin Cancer Res* 2004, **10**:7994–8004.
32. Shimizu M, Yasuda Y, Sakai H, Kubota M, Terakura D, Baba A, Ohno T, Kochi T, Tsurumi H, Tanaka T, Moriwaki H: **Pitavastatin suppresses diethylnitrosamine-induced liver preneoplasms in male C57BL/KsJ-db/db obese mice.** *BMC Cancer* 2011, **11**:281.
33. Kirstein MM, Boukouris AE, Pothiraju D, Buitrago-Molina LE, Marhenke S, Schutt J, Orlik J, Kühnel F, Hegermann J, Manns MP, Vogel A: **Activity of the mTOR**

inhibitor RAD001, the dual mTOR and PI3-kinase inhibitor BEZ235 and the PI3-kinase inhibitor BKM120 in hepatocellular carcinoma. *Liver Int* 2013, **33**:780–793.
34. Shimizu M, Suzui M, Deguchi A, Lim JT, Weinstein IB: **Effects of acyclic retinoid on growth, cell cycle control, epidermal growth factor receptor signaling, and gene expression in human squamous cell carcinoma cells.** *Clin Cancer Res* 2004, **10**:1130–1140.
35. Zhao S, Konopleva M, Cabreira-Hansen M, Xie Z, Hu W, Milella M, Estrov Z, Mills GB, Andreeff M: **Inhibition of phosphatidylinositol 3-kinase dephosphorylates BAD and promotes apoptosis in myeloid leukemias.** *Leukemia* 2004, **18**:267–275.
36. Yamada Y, Shidoji Y, Fukutomi Y, Ishikawa T, Kaneko T, Nakagama H, Imawari M, Moriwaki H, Muto Y: **Positive and negative regulations of albumin gene expression by retinoids in human hepatoma cell lines.** *Mol Carcinog* 1994, **10**:151–158.
37. Alvarez S, Germain P, Alvarez R, Rodriguez-Barrios F, Gronemeyer H, de Lera AR: **Structure, function and modulation of retinoic acid receptor beta, a tumor suppressor.** *Int J Biochem Cell Biol* 2007, **39**:1406–1415.
38. Park EY, Wilder ET, Chipuk JE, Lane MA: **Retinol decreases phosphatidylinositol 3-kinase activity in colon cancer cells.** *Mol Carcinog* 2008, **47**:264–274.
39. Ihle NT, Lemos R Jr, Wipf P, Yacoub A, Mitchell C, Siwak D, Mills GB, Dent P, Kirkpatrick DL, Powis G: **Mutations in the phosphatidylinositol-3-kinase pathway predict for antitumor activity of the inhibitor PX-866 whereas oncogenic Ras is a dominant predictor for resistance.** *Cancer Res* 2009, **69**:143–150.
40. Nakagawa T, Shimizu M, Shirakami Y, Tatebe H, Yasuda I, Tsurumi H, Moriwaki H: **Synergistic effects of acyclic retinoid and gemcitabine on growth inhibition in pancreatic cancer cells.** *Cancer Lett* 2009, **273**:250–256.

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REVIEW

Obesity and hepatocellular carcinoma: targeting obesity-related inflammation for chemoprevention of liver carcinogenesis

Masahito Shimizu · Takuji Tanaka · Hisataka Moriwaki

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Abstract Obesity and related metabolic abnormalities, including a state of chronic inflammation, increase the risk of hepatocellular carcinoma (HCC). Adipose tissue constitutively expresses the proinflammatory cytokine tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), which are important tumor promoters in inflammation-related carcinogenesis. Dysregulation of TNF-α and IL-6 is associated with the development of steatosis and inflammation within the liver. These cytokines also lie at the core of the association between obesity and insulin resistance, which is a key factor in the development of obesity-related HCC. Here we present a detailed review of the relationship between metabolic abnormalities and the development of HCC, focusing on the role played by inflammation. Drawing from our basic and clinical research, the present report also reviews evidence that targeting metabolic abnormalities, such as attenuation of chronic inflammation and improvement of insulin resistance by either pharmaceutical or nutritional intervention, may be an effective strategy in preventing the development of HCC in obese individuals.

Keywords Obesity · Inflammation · Hepatocellular carcinoma · Chemoprevention

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M. Shimizu (✉) · H. Moriwaki
Department of Gastroenterology, Gifu University Graduate School of Medicine,
1-1 Yanagido,
Gifu 501-1194, Japan
e-mail: shimim-gif@umin.ac.jp

T. Tanaka
The Tohkai Cytopathology Institute: Cancer Research and Prevention (TCI-CaRP),
Gifu 500-8285, Japan

Introduction

Obesity, a condition resulting from an excess of adipose tissue, is currently a serious health problem throughout the world, with approximately 1.6 billion overweight and 500 million obese adults [1]. Numerous health disorders complicate obesity, including cardiovascular disease, hypertension, insulin resistance, diabetes mellitus, and hyperlipidemia, which are collectively known as "metabolic syndrome." Non-alcoholic fatty liver disease (NAFLD), which is known to be a hepatic manifestation of metabolic syndrome, is also the most common form of chronic liver disease in developed countries [2, 3]. In addition, recently, obesity and its related metabolic abnormalities, especially diabetes mellitus, have been recognized as major risk factors for the development of certain types of human malignancies, including hepatocellular carcinoma (HCC) [4–16]. A prospective study of a population of more than 900,000 American adults showed that a higher body mass index (BMI) is significantly associated with higher rates of death from cancer, including HCC [17].

Mounting evidence obtained from experimental and epidemiological studies indicates that several pathophysiological mechanisms link obesity and liver carcinogenesis, including the emergence of insulin resistance, alterations in the insulin-like growth factor-1 (IGF-1)/IGF-1 receptor (IGF-1R) axis, a state of chronic inflammation, induction of oxidative stress, and the occurrence of adipokine imbalance [4–8]. Insulin resistance leads to an increased expression of proinflammatory cytokine tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), central mediators of chronic inflammatory diseases, and their dysregulation is associated with the development of steatosis and inflammation within the liver [4–8]. Therefore, among obesity-related pathophysiological conditions that cooperatively enhance the development of HCC, insulin resistance and the subsequent inflammatory cascade are thought to play a critical role in the development of HCC [4–8]. On the other hand, studies

of these conditions also suggest that such pathophysiological disorders might be critical targets for inhibiting obesity-related carcinogenesis [18]. For instance, experimental studies have revealed that improvement of chronic inflammation by inhibiting the expression of TNF-α and IL-6 plays a significant role in the prevention of obesity-related colorectal tumorigenesis [19–21].

The present review aims to summarize multiple pathogenic mechanisms by which obesity and related metabolic disorders influence the development of HCC, focusing on the emergence of insulin resistance and the subsequent inflammatory cascade. This article also aims to review the possibility that nutritional or pharmaceutical approaches targeting pathophysiological conditions caused by obesity might be effective in preventing obesity-related liver carcinogenesis.

Obesity, diabetes mellitus, and HCC

HCC, which is the dominant form of primary liver carcinoma worldwide, is one of the most frequently occurring cancers in the world, accounting for 750,000 annual cases; approximately the same number of individuals (700,000) die from this malignancy each year [22]. Although HCC development is frequently associated with chronic inflammation and subsequent cirrhosis of the liver induced by a persistent infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), recent epidemiological and clinical studies have revealed that obesity and diabetes mellitus are major risk factors for the development of HCC [6–9, 12–16, 23]. In particular, a recent meta-analysis concluded that the summary relative risk of HCC was 117 % for overweight subjects (BMI 25–30 kg/m^2) and 189 % for the obese individuals (BMI ≥30 kg/m^2) [14]. Obesity represents an independent HCC risk factor in patients with alcoholic and cryptogenic cirrhosis [15]. The association between HCC development and diabetes, which is characterized by hyperglycemia, insulin resistance, and hyperinsulinemia, has also been ascertained by repeated meta-analyses [10, 11]. In one population-based study, diabetes increased the risk of HCC by threefold [23]. Insulin resistance has also been shown to raise the risk for recurrence of HCC after curative radiofrequency ablation in HCV-positive patients [13].

The relationship between HCV infection and metabolic syndrome is clinically relevant because insulin resistance and subsequent diabetes and severe steatosis frequently occur in HCV-infected patients [24, 25]. Furthermore, there are synergistic effects between metabolic disorders (obesity and diabetes) and other HCC risk factors such as hepatitis virus infection and alcohol consumption [23, 26–29]. A long-term (14 years) follow-up study in Taiwan has shown that the combined presence of HCV and diabetes is associated with a 37-fold increase in the rate of HCC development [23]. Moreover, HCC risk is increased by more than 100-fold in HBV or HCV carriers with both obesity and diabetes [23]. A recent prospective study showed that insulin resistance itself is associated with HCC in HCV-positive cirrhosis and is a strong predictor of liver-related death or transplantation [30]. Therefore, viral hepatitis patients with metabolic disorders would seem to be at high risk for the development of HCC and thus should be closely monitored for this malignancy.

NAFLD, nonalcoholic steatohepatitis, and HCC

NAFLD is the major hepatic manifestation of obesity and its related metabolic disorders, particularly diabetes mellitus and dyslipidemia, and has become one of the most common liver disorders in developed countries [2, 3, 31, 32]. The accumulation of fat caused by excess energy intake can result in liver dysfunction as the liver synthesizes more triglycerides but fails to export them. Triglyceride deposition in hepatocytes leads to hepatic steatosis. The overlap between the prevalence of NAFLD and diabetes is equally substantial [32]. On the other hand, NAFLD is commonly associated with insulin resistance and hyperinsulinemia even in the non-obese [33], indicating that insulin resistance might be a key factor in the development of NAFLD. In addition, NAFLD that has not yet progressed to nonalcoholic steatohepatitis (NASH) can induce hepatocyte proliferation and hepatic hyperplasia, both of which initiate the hepatic neoplastic process in obesity [34].

While most patients with NAFLD remain asymptomatic, 20 % progress to develop chronic hepatic inflammation or NASH, which in turn can lead to liver fibrosis, portal hypertension, cirrhosis, HCC development, and increased mortality [2, 3, 31, 32, 35]. A subsequent study of natural history in NAFLD indicates that steatohepatitis is a risk for the development of cirrhosis and HCC [36]. The exact prevalence of HCC in NASH remains unknown; however, some prospective studies found at least 2 to 3 % yearly cumulative incidence of HCC in patients with NASH [37, 38]. In 1998, Day and James proposed a "two-hit theory" to explain NAFLD/NASH pathogenesis [39]. The first hit, the flux of free fatty acids into the liver and subsequent hepatic steatosis, plays a role in lipotoxicity-induced mitochondrial abnormalities that sensitize the liver to additional proinflammatory insults, the second hit. These hits include enhanced lipid peroxidation and increased generation of reactive oxygen species. Insulin resistance is also regarded as a critical factor in the etiology of NASH [39, 40].

Potential pathophysiological mechanisms linking obesity and HCC development

Figure 1 shows several pathophysiological mechanisms linking obesity and its related metabolic abnormalities to liver carcinogenesis. Substantial evidence has shown that insulin resistance, among various obesity-related metabolic disorders, significantly contributes to the development of HCC. Insulin, which is a key regulator of glucose metabolism itself, and the signal transduction network it regulates play important roles in oncogenesis [41, 42]. Insulin induces HCC cells to proliferate and resist apoptosis [43, 44], suggesting that hyperinsulinemia directly contributes to the growth of HCC cells. In addition, insulin resistance increases the biological activity of IGF-1, an important endocrine and paracrine regulator of tissue growth and metabolism. Numerous pieces of evidence indicate that the IGF-1/IGF-1R axis plays an important role in the carcinogenesis of many cancer types, including HCC [41, 42]. Insulin receptor and IGF-1R are receptor tyrosine kinases, and the binding of insulin and IGF-1 to their respective receptors on tumors and precancerous cells activates the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, which is responsible for cellular processes like growth, proliferation, and survival [41, 42]. IGF-1R activity is also required for oncogenic transformation by a number of oncogenes, including RAS, and can promote tumor formation in vivo [41, 45]. Activation of the IGF/IGF-1R axis is critically involved in the growth of HCC cells and in liver carcinogenesis [46–48]. For HCC, IGF-1R activation is observed in a subgroup of tumor cells but not in adjacent cirrhotic tissue [48]. We have recently reported that insulin resistance and the activation of IGF/IGF-1R axis are involved in liver carcinogen *N*-diethylnitrosamine (DEN)-induced liver tumorigenesis in obese and diabetic C57BL/KsJ-*db*/*db* (*db*/*db*) mice [49, 50].

An adipokine imbalance caused by excess production of storage lipids may also be related to obesity-associated liver carcinogenesis. For instance, higher levels of serum leptin, which regulates energy homeostasis and is elevated in obese individuals [51], increase the risk of HCC recurrence after curative treatment [52]. Leptin stimulates the growth of HCC cells by upregulating cyclin D1 expression [53]. Treatment with leptin also increases the proliferation of HCC-derived cells by activating several signaling pathways: signal transducer and activator of transcription-3 (Stat3), AKT, and extracellular signal-regulated kinase (ERK) [54]. In animal models, leptin has been shown to promote angiogenesis and thus could facilitate the progression of NASH to HCC [55]. In addition, lack of adiponectin, the other member of the adipokine group that is significantly reduced in obese individuals [56], enhances the progression of hepatic

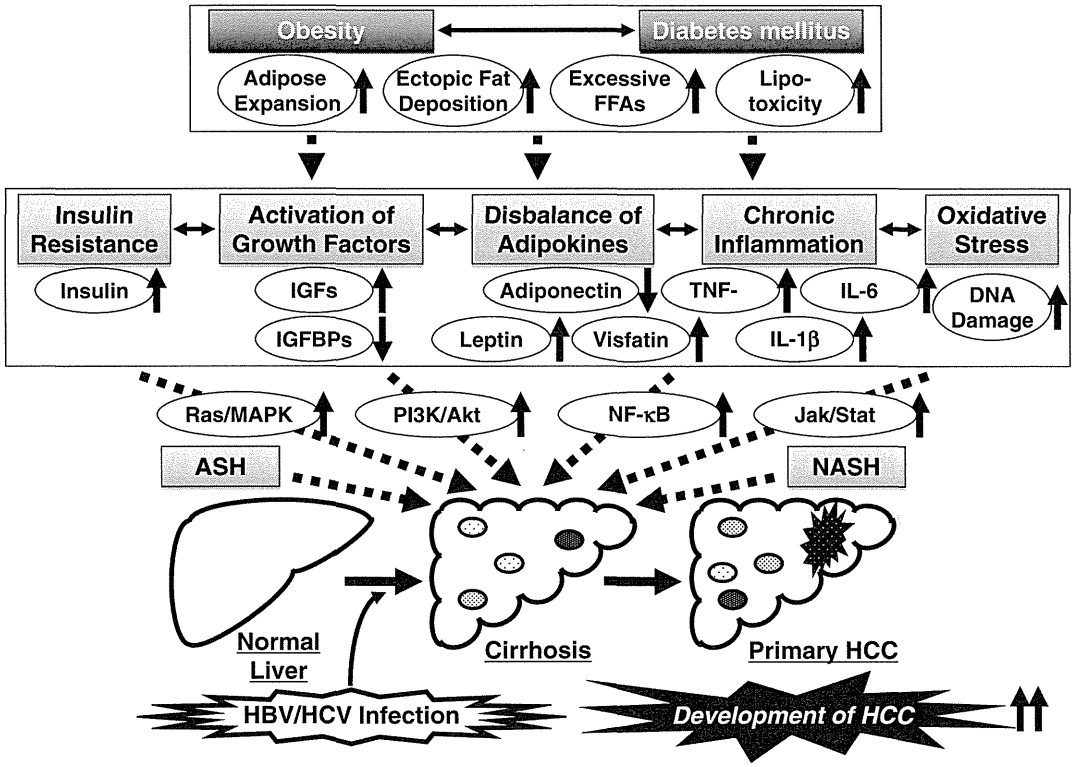

Fig. 1 Proposed mechanisms linking obesity and its related metabolic abnormalities to the development of HCC

steatosis and tumor formation in a mouse model of NASH [57]. However, this adipokine alleviates hepatic steatosis [58]. In vitro and in vivo studies show that adiponectin exerts antitumor effects in HCC cells [59]. Moreover, the induction of adiponectin plays a role in the suppression of chemically induced liver tumorigenesis in obese mice [60]. These findings suggest that obesity and its related metabolic abnormalities, such as sustained insulin resistance, activation of the IGF-1/IGF-1R axis, and adipokine imbalance, play an important role in the development of HCC and thus might be promising targets in the prevention of obesity-related liver tumorigenesis.

Obesity-induced insulin resistance and chronic inflammation

There is substantial evidence that obesity is associated with chronic low-grade systemic inflammation, which contributes to metabolic disorders and the progression from hepatic steatosis to NASH and subsequent HCC development [4–8]. Hypertrophic adipocytes, which are associated with the deposition and accumulation of excess lipids, secrete free fatty acids (FFAs); in addition, together with various immune cells, they release various proinflammatory cytokines, including TNF-α and IL-6 [4–8]. In particular, macrophage infiltration into white adipose tissue, which is accompanied by TNF-α and IL-6 production, is an early contributing event for the development of chronic low-grade systemic inflammation [61, 62]. In 1993, Hotamisligil et al. demonstrated that adipocytes constitutively express TNF-α and neutralization of TNF-α by soluble TNF-α receptor decreases insulin resistance in obese mice [63]. This suggests that TNF-α lies at the core of the association between obesity and insulin resistance. TNF-α enhances obesity-related systemic insulin resistance by inhibiting the tyrosine phosphorylation of insulin receptor [64]. On the other hand, the loss of TNF-α and its receptor improves insulin sensitivity in obese mice [65]. TNF-α contributes to obesity-induced IL-6 production, which causes hepatic inflammation and activates ERK and Stat3 [66]. TNF-α and IL-6 expressions in the liver are strongly induced in response to a high-fat diet, but inhibition of TNF-α signaling or ablation of IL-6 prevents hepatosteatosis [66]. Type 2 diabetes is an inflammatory condition, as evidenced by the elevated concentrations of IL-6, which induces cellular insulin resistance in hepatocytes, observed in these patients [67–69]. The concentration of IL-6 together with IL-1β, which is another inflammatory cytokine that induces insulin resistance in liver-derived cells, is a more predictive risk factor for type 2 diabetes in humans than either cytokine alone [70, 71]. TNF-α and IL-6 increase the levels of leptin, whereas leptin influences inflammatory responses, possibly by triggering the release of TNF-α and IL-6 [72, 73]. Hepatic steatosis has negative effects on liver function, which might be mediated by inflammation because the expression of TNF-α, IL-6, and IL-1β mRNA increases in the liver with increasing adiposity [74].

Cytokine signaling pathway associated with obesity-induced inflammation and HCC development

Several specific intracellular signaling pathways, including c-Jun N-terminal kinase (JNK) and nuclear factor (NF)-κB, have emerged as potential targets for many inflammatory cytokines and chemokines that promote obesity-related metabolic disorders such as insulin resistance [75]. For instance, activation of JNK inhibits normal tyrosine phosphorylation of insulin receptor substrate-1 and downstream insulin signal transduction [76]. The effects of obesity-induced activation of NF-κB are mediated through the synthesis of NF-κB target gene expression, including TNF-α, IL-6, and IL-1β [77]. Therefore, activation of JNK and NF-κB is associated with the induction of insulin resistance, whereas their inhibition provides glucose tolerance and protection from obesity in rodents [75]. Reactive oxygen species that are increased by adiposity have also been shown to activate JNK and NF-κB [78]. In addition, saturated FFAs lead to JNK activation, which can, in turn, increase the production of inflammatory cytokines capable of causing insulin resistance [79]. Saturated FFAs have also been found to enhance NF-κB activation in macrophages [80], suggesting that there is a potential link between elevated circulating or tissue lipid concentrations and the part of the immune system that mediates inflammation. In hepatocytes, saturated FFAs can induce time- and dose-dependent lipoapoptosis, which is the combination of lipid accumulation and induction of apoptosis in hepatocytes [81]. Experimental data have also shown that FFAs cause TNF-α production and subsequent NF-κB activation by promoting hepatic lipotoxicity [82]. These findings appear significant because lipotoxicity and lipoapoptosis play a pivotal role in the progression of NAFLD to NASH [83]. JNK1 activation also promotes the development of NASH in mice fed with methionine- and choline-deficient diets [84], which indicates that JNK and NF-κB are critical factors in the occurrence of NAFLD and its progression to NASH.

The role of obesity-induced inflammation in liver tumorigenesis has recently been demonstrated in several experimental models [50, 66, 85, 86]. For instance, administration of DEN was found to enhance the development of preneoplastic lesions in the livers of rats fed with high-fat diets and this was associated with elevated TNF-α/NF-κB signaling and ERK-related hepatocyte proliferation [85]. Phosphorylation of ERK, Akt, Stat3, and JNK proteins and upregulation of

TNF-α, IL-6, and IL-1β in the liver are involved in DEN-induced liver tumorigenesis in *db/db* obese mice [50]. Enhanced production of adipose-derived TNF-α and IL-6 and activation of Stat3 are critical in the development of obesity-related liver tumorigenesis [66]. This study [66], together with another recent study [87], clearly indicates that Stat3 activation, which is associated with TNF-α and IL-6 production in hepatocytes, is essential for liver carcinogenesis.

Targeting obesity-related metabolic abnormalities for cancer prevention

As mentioned earlier, obesity and its related metabolic abnormalities, such as a state of chronic inflammation, play a critical role in the development of HCC. On the other hand, these findings may suggest the possibility that the metabolic disorders caused by obesity might be effective targets in the prevention of liver carcinogenesis [18]. For instance, ablation of IL-6 or inhibition of TNF-α signaling can inhibit obesity-promoted hepatocarcinogenesis by reducing hepatosteatosis and steatohepatitis [66]. Treatment with adiponectin, an anti-inflammatory adipokine, also reduces liver tumorigenesis in nude mice [59].

To verify our hypothesis that targeting metabolic abnormalities caused by obesity might be an effective strategy for preventing cancer development in obese individuals, we have conducted several experimental studies. We initially performed chemopreventive studies using a mouse model of obesity-related colorectal carcinogenesis because increased body fat levels and BMI are associated with an increased risk of colorectal cancer [17, 88, 89]. The model used obese and diabetic *db/db* mice, which are susceptible to the colonic carcinogen azoxymethane (AOM) and thus easily develop colonic precancerous lesions [90]. We have found that pitavastatin and renin–angiotensin system inhibitors, which are drugs for hyperlipidemia and hypertension, respectively, suppress AOM-induced colonic preneoplastic lesions in *db/db* mice by inhibiting the levels of TNF-α and IL-6 in the serum and colonic mucosa [20, 21]. Curcumin, a component of turmeric, also exerts chemopreventive effects in the development of obesity-related colonic preneoplastic lesions in *db/db* mice, and this is associated with inhibition of NF-κB activity and TNF-α and IL-6 expression in the colonic mucosa [19]. Furthermore, branched-chain amino acids (BCAA) and (−)-epigallocatechin gallate (EGCG) prevent obesity-related colorectal carcinogenesis by improving insulin resistance and inhibiting IGF/IGF-1R axis in these mice [91, 92].

Among these agents, BCAA is considered as one of the most promising candidates to prevent obesity-related liver tumorigenesis. This is because it is widely used for the treatment of protein energy malnutrition (PEM) that frequently occurs in patients with liver cirrhosis [93–96]. EGCG, a major biologically active component of green tea, also seems to have a considerable effect given that green tea catechins (GTCs) improve metabolic abnormalities and possess anticancer and cancer chemopreventive properties [97–100]. In the following sections, we will discuss in detail the effects of BCAA and EGCG in the prevention of obesity-related liver tumorigenesis based on our recent experimental studies. In addition, we also discuss the effects of acyclic retinoid (ACR), which is a promising agent for the chemoprevention of HCC [101–104], on the prevention of liver tumorigenesis in obese mice.

Preventive effects of BCAA on obesity-related liver tumorigenesis

Because the liver is a critical organ for regulating metabolism, a variety of nutritional and metabolic disorders, such as PEM and insulin resistance, are frequently seen in patients with chronic liver diseases [93–96, 105, 106]. Decreased serum levels of BCAA (valine, leucine, and isoleucine) and albumin appear with a high incidence in liver cirrhosis, whereas supplementation with BCAA has been shown to improve PEM and increase the serum albumin concentration in cirrhotic patients. This subsequently improves the quality of life and prognosis in patients with liver cirrhosis by preventing complications associated with the disease [93–96]. In addition, recent clinical and experimental studies have revealed that BCAA improves insulin resistance and glucose tolerance [107–110]. In 2005, Muto et al. reported the results of a large-scale (n=622) multicenter randomized controlled trial, the Long-Term Survival Study, which investigated the effects of supplemental BCAA therapy on event-free survival in patients with decompensated cirrhosis. In the trial, oral supplementation with a BCAA preparation significantly prevented progressive hepatic failure and improved event-free survival [95], strongly suggesting that supplementation with BCAA can serve as a first-line therapy for patients with decompensated cirrhosis.

Moreover, it should be emphasized that the results of the subset analysis from this trial demonstrated that long-term oral supplementation with BCAA was associated with a reduced frequency of HCC in obese cirrhotic patients (P= 0.008) [12]. To clarify the precise mechanisms of BCAA in the prevention of the development of HCC in obese cirrhotic patients, we performed an experimental study using the obesity-related liver carcinogenesis model in *db/db* mice [49]. In the study, BCAA supplementation significantly suppressed the development of DEN-induced hepatic preneoplastic lesions in *db/db* mice by inhibiting the expression of IGF-1, IGF-2, and IGF-1R in the liver. The development of liver neoplasms, including hepatic adenoma and HCC,

was also reduced by BCAA supplementation, and this was associated with improvement of insulin resistance, reduction of serum leptin levels, and attenuation of hepatic steatosis and fibrosis [49]. Obese cirrhotic patients generally have a particularly high incidence of hyperinsulinemia and insulin resistance [105, 106]. Therefore, our findings [49], together with the results of an in vitro study showing that BCAA suppresses insulin-induced proliferation of HCC cells by inhibiting the insulin-induced activation of the PI3K/Akt pathway [111], suggest that BCAA supplementation reduced the risk of developing HCC in obese cirrhotic patients. This was accomplished, at least in part, by targeting insulin resistance and its related signaling pathways (Fig. 2; Table 1). These findings are consistent with the results of an experimental study reported by Yoshiji et al. showing the chemopreventive effects of BCAA supplementation against liver tumorigenesis in obese and diabetic rats, which are also complicated with insulin resistance [112].

In addition, in our unpublished study, BCAA supplementation was shown to suppress the spontaneous development of hepatic preneoplastic lesions in *db/db* mice by inhibiting the expression of TNF-α, IL-6, and IL-1β mRNA in the liver. BCAA supplementation also inhibited increased macrophage infiltration and the expression of TNF-α, IL-6, and monocyte chemoattractant protein-1 mRNA in the white adipose tissue, suggesting that chronic inflammation induced by obesity in the liver and adipose tissue could also serve as a critical target of BCAA in the inhibition of the early phase of obesity-related liver tumorigenesis (unpublished data).

Preventive effects of GTCs on obesity-related liver tumorigenesis

Green tea is a beverage commonly consumed worldwide. Its component polyphenols, which are known as GTCs, have received great attention for their beneficial effects, particularly their involvement in the improvement of metabolic abnormalities and prevention of certain types of malignancies [97–100]. A recent meta-analysis of clinical trials reported that GTCs help reduce body weight [98]. Supplementation with GTCs was found to decrease plasma levels of insulin, TNF-α, and IL-6 and improve hepatic steatosis and liver dysfunction in a rodent model of obesity and diabetes. This indicated that treatment with GTCs is effective in the prevention of the progression of obesity-related metabolic disorders such as chronic inflammation [113–115]. The anti-inflammatory properties of GTCs are also responsible for the anticancer and cancer-preventive effects of the molecules [99]. EGCG, a type of GTC, suppresses inflammation-related colon carcinogenesis in mice by decreasing the mRNA expression of TNF-α and IL-6 in the colonic mucosa [116]. EGCG also inhibits proliferation and induces apoptosis in HCC- and colorectal cancer-derived cells by inhibiting the activation of IGF-1R and its

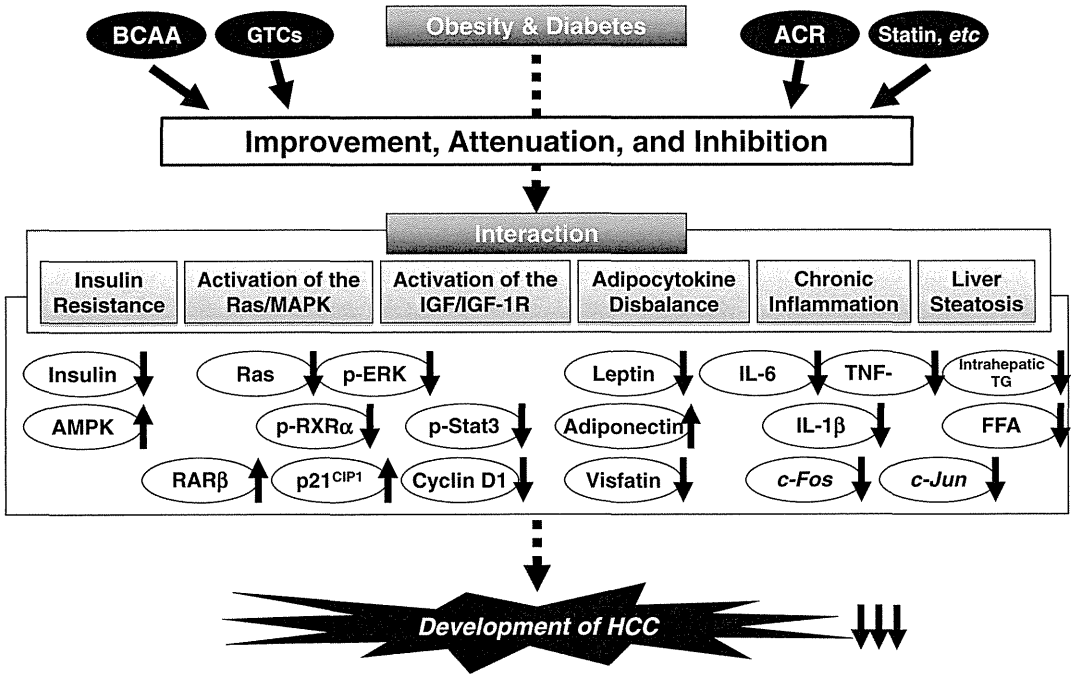

Fig. 2 Mechanisms of action of BCAA, EGCG, and ACR in the inhibition of obesity-related liver carcinogenesis

Table 1 Suppressive effects of BCAA, EGCG, ACR, and pitavastatin on obesity-related liver tumorigenesis in *db/db* mice

Agent	Inhibition rate (%)		Inhibition mechanisms		Reference number
	Adenoma	FCA[a]			
BCAA	75[b, c]	50[b, c]	Hepatic IGF-1, IGF-2, and IGF-1R mRNAs ↓ Hepatic steatosis ↓	Serum leptin and ALT levels ↓ Insulin sensitivity ↑	[49]
			Hepatic fibrosis ↓	Hepatocyte proliferation ↓	
EGCG	86[c]	48[c]	Hepatic pIGF-1R, pERK, and pAkt proteins ↓ Hepatic steatosis ↓	Serum insulin, IGF-1, IGF-2, and FFA levels ↓ Hepatic pAMPK protein ↑	[50]
			Hepatic and systemic inflammation ↓	Hepatic pStat3 and pJNK proteins ↓	
ACR	86[c]	81[c]	Hepatic Ras activity ↓	Hepatic pRXRα, pERK, and pStat3 proteins ↓	[86]
			Hepatic RARβ and $p21^{CIP1}$ mRNAs ↑	Hepatic steatosis ↓	
			Insulin sensitivity ↑	Hepatic and systemic inflammation ↓	
Pitavastatin	NE[d]	29[e]	Pro-apoptotic effect ↑ Hepatic steatosis ↓	Hepatocyte proliferation ↓ Hepatic pAMPK protein ↑	[60]
			Serum adiponectin level ↑	Hepatic and systemic inflammation ↓	

[a] Foci of cellular alteration

[b] Compared to the casein supplementation mice (a nitrogen content-matched control for BCAA)

[c] Mice were treated with agent for 34 weeks

[d] Not examined

[e] Mice were treated with agent for 14 weeks

downstream signaling pathways, including Ras/MAPK and PI3K/Akt [46, 117]. In addition, this agent prevents carbon tetrachloride-induced hepatic fibrosis in rats by inhibiting IGF-1R expression [118], indicating that the IGF/IGF-1R axis, which is critically involved in cancer development and obesity-related metabolic disorder, might be a critical target of GTCs. Several interventional studies also provide clear evidence for the chemopreventive effects and safety of tea preparations [119–121].

Because GTCs are expected to improve metabolic disorders and exert chemopreventive properties by targeting chronic inflammation and the IGF/IGF-1R axis, we examined whether EGCG treatment inhibits obesity-associated liver tumorigenesis [50]. We found that drinking water containing EGCG significantly inhibited the development of hepatic preneoplastic lesions and adenoma [50]. EGCG consumption also improved hepatic steatosis; decreased the serum levels of insulin, IGF-1, and IGF-2; and inhibited the phosphorylation of the IGF-1R, ERK, Akt, Stat3, and JNK proteins in the liver of obese mice [50]. The serum levels of FFA and TNF-α were also decreased by drinking EGCG, which additionally lowered the expression of TNF-α, IL-6, and IL-1β mRNAs in the liver [50]. These findings suggest that EGCG prevents obesity-related liver tumorigenesis by inhibiting the IGF/IGF-1R axis, improving hyperinsulinemia, and attenuating chronic inflammation (Fig. 2; Table 1). Thus, in addition to BCAA, GTCs may also be useful in the chemoprevention of liver tumorigenesis in obese individuals.

Preventive effects of ACR on obesity-related liver tumorigenesis

Retinoids, a group of structural and functional derivatives of vitamin A, play fundamental roles in cellular activities, including growth, differentiation, and apoptosis, as well as in morphology [122, 123]. Because of this, loss of retinoid activity or responsiveness is linked to the development of several types of human malignancies, including HCC; therefore, they might be critical targets for cancer chemoprevention and chemotherapy [103, 104, 124, 125]. Retinoids exert their biological functions primarily by regulating gene expression through two distinct nuclear receptors, the retinoic acid receptors (RARs) and retinoid X receptors (RXRs), both of which are composed of three subtypes (α, β, and γ) [122, 123]. Among the retinoid receptors, RXRα is thought to be one of the most important with respect to exerting fundamental effects on cellular activities. This is because it forms a heterodimer with other nuclear receptors and thereby acts as the master regulator of nuclear receptors [122, 123]. We have reported that abnormalities in the expression and function of RXRα are prominently involved in the development of HCC. The repression of RXRα was found to occur in the early stages of liver carcinogenesis in a rat model of chemically induced liver carcinogenesis [126]. Moreover, a malfunction of the RXRα due to phosphorylation by the Ras/MAPK signaling pathway is significantly

associated with liver carcinogenesis. That is, accumulation of phosphorylated RXRα protein, which is regarded as the nonfunctional form of RXRα, interferes with the function of normal (unphosphorylated) RXRα in a dominant-negative manner, thus playing a critical role in HCC development [103, 104, 127–130]. These findings therefore suggest that targeting RXRα phosphorylation may be an effective and important strategy for the prevention and treatment of HCC.

ACR, a synthetic retinoid that was initially developed as an agonist for RXR, is a possible candidate for this purpose because it can impede the development of HCC and it inhibits cancer cell growth by repressing the Ras/MAPK signaling pathway and subsequent RXRα phosphorylation [103, 104, 128, 131]. One early-phase randomized controlled clinical trial tested the chemopreventive effect of ACR on secondary HCC in patients who underwent potentially curative treatment for initial HCC. In this study, oral administration of ACR significantly reduced the incidence of recurrent or new HCC (P=0.04) and improved the recurrence-free survival (P=0.002) and overall survival rates (P=0.04) [101, 102]. Moreover, a large-scale (n=401) randomized placebo-controlled trial (phase II/III trial) also showed that ACR had a strong effect on the prevention of second primary HCC in HCV-positive patients. It showed a hazard ratio of 0.27 (95 % CI, 0.07–0.96) 2 years after the treatment, indicating that ACR reduced the recurrence of HCC, particularly after 2 years of treatment [132].

Because numerous preclinical experiments and clinical trials indicate that ACR is a promising agent for the chemoprevention of HCC, we investigated whether ACR could prevent obesity-related liver tumorigenesis [86]. In the study, treatment with ACR effectively prevented the development of obesity-related liver tumorigenesis by inhibiting the activation of Ras and the phosphorylation of ERK and RXRα, thus restoring RXRα function in the liver of DEN-treated *db/db* mice [86]. ACR administration also inhibits this tumorigenesis through attenuation of the chronic inflammation induced by excessive fatty deposits, as demonstrated by the improved liver steatosis and decreased serum TNF-α levels and expression levels of TNF-α, IL-6, and IL-1β mRNA in the liver [86]. In addition, ACR administration improved insulin sensitivity, which was also associated with the prevention of obesity-related liver tumorigenesis [86] (Fig. 2; Table 1). Therefore, the results obtained from both clinical trials [101, 102, 132] and this preclinical experiment [86] encourage the clinical use of ACR for cirrhotic patients with obesity and diabetes who are at a notably higher risk of developing HCC.

Conclusion

Obesity and its related metabolic abnormalities, including increased cancer risk, are a serious public health problem worldwide. Among all cancers, HCCs are the malignancies most frequently affected by obesity. The liver disease influenced most by obesity is NAFLD, and this disease, by itself and in synergy with other risk factors such as hepatitis virus infection, is becoming one of the most common causes of HCC in developed countries. Therefore, there is an urgent need to develop more effective therapeutic strategies to prevent the development of obesity-related HCC or halt its progression. Obesity and diabetes enhance HCC development through insulin resistance, activation of the IGF/IGF-1R axis, and lipid accumulation within hepatocytes, thereby leading to a chronic low-grade systemic inflammation. This involves abnormalities of various types of cytokines and adipokines. Among them, TNF-α and IL-6 play a critical role in the onset of NASH and the initiation and promotion of HCC.

In this review, we indicate the possibility that pharmaceutical and nutraceutical approaches for targeting and restoring metabolic disorders, especially chronic low-grade inflammation involving increased levels of TNF-α and IL-6, may be an effective strategy for preventing the development of obesity-related HCC. We further indicate that BCAA, GTCs, and ACR are considered as some of the most promising agents for achieving this purpose. Therefore, further advanced translational research, such as pilot trials, to clarify whether active intervention using these agents can prevent the development and recurrence of HCC in patients with chronic liver disease and obesity is required. In addition, further experimental studies to determine whether specific drugs, such as antidiabetic drugs, antihypertensive drugs, and lipid-lowering drugs, can inhibit obesity-related liver carcinogenesis should be performed. Considering that these drugs are widely used for patients with metabolic syndrome, it would be beneficial if they could exert chemopreventive effects on obesity-associated carcinogenesis. Our recent findings that pitavastatin, a recently developed lipophilic statin, suppresses the development of chemically induced colonic and hepatic preneoplastic lesions in *db/db* mice by attenuating chronic inflammation may provide a basis for this attempt [21, 60] (Fig. 2; Table 1).

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References

1. WHO: World Health Organization fact sheet for world wide prevalence of obesity. http://wwwwhoint/mediacentre/factsheets/fs311/en/indexhtml. Accessed on 27 December 2011
2. Angulo P (2002) Nonalcoholic fatty liver disease. N Engl J Med 346(16):1221–1231
3. Siegel AB, Zhu AX (2009) Metabolic syndrome and hepatocellular carcinoma: two growing epidemics with a potential link. Cancer 115(24):5651–5661
4. Calle EE, Kaaks R (2004) Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer 4(8):579–591
5. Anderson AS, Caswell S (2009) Obesity management—an opportunity for cancer prevention. Surgeon 7(5):282–285
6. Sun B, Karin M (2012) Obesity, inflammation, and liver cancer. J Hepatol 56(3):704–713
7. El-Serag HB, Rudolph KL (2007) Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 132(7):2557–2576
8. El-Serag HB, Tran T, Everhart JE (2004) Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. Gastroenterology 126(2):460–468
9. Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB (2005) Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. Gut 54 (4):533–539
10. El-Serag HB, Hampel H, Javadi F (2006) The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. Clin Gastroenterol Hepatol 4(3):369–380
11. Wang P, Kang D, Cao W, Wang Y, Liu Z (2012) Diabetes mellitus and risk of hepatocellular carcinoma: a systematic review and meta-analysis. Diabetes Metab Res Rev 28(2):109–122
12. Muto Y, Sato S, Watanabe A, Moriwaki H, Suzuki K, Kato A, Kato M, Nakamura T, Higuchi K, Nishiguchi S, Kumada H, Ohashi Y, Long-Term Survival Study Group (2006) Overweight and obesity increase the risk for liver cancer in patients with liver cirrhosis and long-term oral supplementation with branched-chain amino acid granules inhibits liver carcinogenesis in heavier patients with liver cirrhosis. Hepatol Res 35(3):204–214
13. Imai K, Takai K, Nishigaki Y, Shimizu S, Naiki T, Hayashi H, Uematsu T, Sugihara J, Tomita E, Shimizu M, Nagaki M, Moriwaki H (2010) Insulin resistance raises the risk for recurrence of stage I hepatocellular carcinoma after curative radiofrequency ablation in hepatitis C virus-positive patients: a prospective, case series study. Hepatol Res 40(4):376–382
14. Larsson SC, Wolk A (2007) Overweight, obesity and risk of liver cancer: a meta-analysis of cohort studies. Br J Cancer 97 (7):1005–1008
15. Nair S, Mason A, Eason J, Loss G, Perrillo RP (2002) Is obesity an independent risk factor for hepatocellular carcinoma in cirrhosis? Hepatology 36(1):150–155
16. Regimbeau JM, Colombat M, Mognol P, Durand F, Abdalla E, Degott C, Degos F, Farges O, Belghiti J (2004) Obesity and diabetes as a risk factor for hepatocellular carcinoma. Liver Transpl 10(2 Suppl 1):S69–S73
17. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ (2003) Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med 348(17):1625–1638
18. Shimizu M, Kubota M, Tanaka T, Moriwaki H (2012) Nutraceutical approach for preventing obesity-related colorectal and liver carcinogenesis. Int J Mol Sci 13(1):579–595
19. Kubota M, Shimizu M, Sakai H, Yasuda Y, Terakura D, Baba A, Ohno T, Tsurumi H, Tanaka T, Moriwaki H (2012) Preventive effects of curcumin on the development of azoxymethane-induced colonic preneoplastic lesions in male C57BL/KsJ-db/db obese mice. Nutr Cancer 64(1):72–79
20. Kubota M, Shimizu M, Sakai H, Yasuda Y, Ohno T, Kochi T, Tsurumi H, Tanaka T, Moriwaki H (2011) Renin–angiotensin system inhibitors suppress azoxymethane-induced colonic preneoplastic lesions in C57BL/KsJ-db/db obese mice. Biochem Biophys Res Commun 410(1):108–113
21. Yasuda Y, Shimizu M, Shirakami Y, Sakai H, Kubota M, Hata K, Hirose Y, Tsurumi H, Tanaka T, Moriwaki H (2010) Pitavastatin inhibits azoxymethane-induced colonic preneoplastic lesions in C57BL/KsJ-db/db obese mice. Cancer Sci 101(7):1701–1707
22. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. CA Cancer J Clin 61(2):69–90
23. Chen CL, Yang HI, Yang WS, Liu CJ, Chen PJ, You SL, Wang LY, Sun CA, Lu SN, Chen DS, Chen CJ (2008) Metabolic factors and risk of hepatocellular carcinoma by chronic hepatitis B/C infection: a follow-up study in Taiwan. Gastroenterology 135 (1):111–121
24. Kaddai V, Negro F (2011) Current understanding of insulin resistance in hepatitis C. Expert Rev Gastroenterol Hepatol 5 (4):503–516
25. Negro F (2010) Abnormalities of lipid metabolism in hepatitis C virus infection. Gut 59(9):1279–1287
26. Hassan MM, Hwang LY, Hatten CJ, Swaim M, Li D, Abbruzzese JL, Beasley P, Patt YZ (2002) Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis and diabetes mellitus. Hepatology 36(5):1206–1213
27. Veldt BJ, Chen W, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, de Knegt RJ, Zeuzem S, Manns MP, Hansen BE, Schalm SW, Janssen HL (2008) Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. Hepatology 47(6):1856–1862
28. Wang CS, Yao WJ, Chang TT, Wang ST, Chou P (2009) The impact of type 2 diabetes on the development of hepatocellular carcinoma in different viral hepatitis statuses. Cancer Epidemiol Biomarkers Prev 18(7):2054–2060
29. Lai SW, Chen PC, Liao KF, Muo CH, Lin CC, Sung FC (2012) Risk of hepatocellular carcinoma in diabetic patients and risk reduction associated with anti-diabetic therapy: a population-based cohort study. Am J Gastroenterol 107(1):46–52
30. Nkontchou G, Bastard JP, Ziol M, Aout M, Cosson E, Ganne-Carrie N, Grando-Lemaire V, Roulot D, Capeau J, Trinchet JC, Vicaut E, Beaugrand M (2010) Insulin resistance, serum leptin, and adiponectin levels and outcomes of viral hepatitis C cirrhosis. J Hepatol 53(5):827–833
31. Byrne CD, Olufadi R, Bruce KD, Cagampang FR, Ahmed MH (2009) Metabolic disturbances in non-alcoholic fatty liver disease. Clin Sci 116(7):539–564
32. Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA (2011) Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology 140(1):124–131
33. Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N (1999) Association of nonalcoholic fatty liver disease with insulin resistance. Am J Med 107(5):450–455
34. Yang S, Lin HZ, Hwang J, Chacko VP, Diehl AM (2001) Hepatic hyperplasia in noncirrhotic fatty livers: is obesity-related hepatic steatosis a premalignant condition? Cancer Res 61(13):5016–5023
35. Starley BQ, Calcagno CJ, Harrison SA (2010) Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. Hepatology 51(5):1820–1832

36. Adams LA, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, Angulo P (2005) The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology 129(1):113–121
37. Ascha MS, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN (2010) The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. Hepatology 51(6):1972–1978
38. Yatsuji S, Hashimoto E, Tobari M, Taniai M, Tokushige K, Shiratori K (2009) Clinical features and outcomes of cirrhosis due to non-alcoholic steatohepatitis compared with cirrhosis caused by chronic hepatitis C. J Gastroenterol Hepatol 24 (2):248–254
39. Day CP, James OF (1998) Steatohepatitis: a tale of two "hits"? Gastroenterology 114(4):842–845
40. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN (2001) Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology 120(5):1183–1192
41. Pollak M (2008) Insulin and insulin-like growth factor signalling in neoplasia. Nat Rev Cancer 8(12):915–928
42. Clayton PE, Banerjee I, Murray PG, Renehan AG (2011) Growth hormone, the insulin-like growth factor axis, insulin and cancer risk. Nat Rev Endocrinol 7(1):11–24
43. Kang S, Song J, Kang H, Kim S, Lee Y, Park D (2003) Insulin can block apoptosis by decreasing oxidative stress via phosphatidylinositol 3-kinase- and extracellular signal-regulated protein kinase-dependent signaling pathways in HepG2 cells. Eur J Endocrinol 148(1):147–155
44. Tornkvist A, Parpal S, Gustavsson J, Stralfors P (1994) Inhibition of Raf-1 kinase expression abolishes insulin stimulation of DNA synthesis in H4IIE hepatoma cells. J Biol Chem 269(19):13919–13921
45. Sell C, Rubini M, Rubin R, Liu JP, Efstratiadis A, Baserga R (1993) Simian virus 40 large tumor antigen is unable to transform mouse embryonic fibroblasts lacking type 1 insulin-like growth factor receptor. Proc Natl Acad Sci U S A 90(23):11217–11221
46. Shimizu M, Shirakami Y, Sakai H, Tatebe H, Nakagawa T, Hara Y, Weinstein IB, Moriwaki H (2008) EGCG inhibits activation of the insulin-like growth factor (IGF)/IGF-1 receptor axis in human hepatocellular carcinoma cells. Cancer Lett 262:10–18
47. Alexia C, Fallot G, Lasfer M, Schweizer-Groyer G, Groyer A (2004) An evaluation of the role of insulin-like growth factors (IGF) and of type-I IGF receptor signalling in hepatocarcinogenesis and in the resistance of hepatocarcinoma cells against drug-induced apoptosis. Biochem Pharmacol 68(6):1003–1015
48. Tovar V, Alsinet C, Villanueva A, Hoshida Y, Chiang DY, Sole M, Thung S, Moyano S, Toffanin S, Minguez B, Cabellos L, Peix J, Schwartz M, Mazzaferro V, Bruix J, Llovet JM (2010) IGF activation in a molecular subclass of hepatocellular carcinoma and pre-clinical efficacy of IGF-1R blockage. J Hepatol 52 (4):550–559
49. Iwasa J, Shimizu M, Shiraki M, Shirakami Y, Sakai H, Terakura Y, Takai K, Tsurumi H, Tanaka T, Moriwaki H (2010) Dietary supplementation with branched-chain amino acids suppresses diethylnitrosamine-induced liver tumorigenesis in obese and diabetic C57BL/KsJ-db/db mice. Cancer Sci 101(2):460–467
50. Shimizu M, Sakai H, Shirakami Y, Yasuda Y, Kubota M, Terakura D, Baba A, Ohno T, Hara Y, Tanaka T, Moriwaki H (2011) Preventive effects of (−)-epigallocatechin gallate on diethylnitrosamine-induced liver tumorigenesis in obese and diabetic C57BL/KsJ-db/db mice. Cancer Prev Res 4(3):396–403
51. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kern PA, Friedman JM (1995) Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med 1(11):1155–1161
52. Watanabe N, Takai K, Imai K, Shimizu M, Naiki T, Nagaki M, Moriwaki H (2011) Increased levels of serum leptin are a risk factor for the recurrence of stage I/II hepatocellular carcinoma after curative treatment. J Clin Biochem Nutr 49(3):153–158
53. Chen C, Chang YC, Liu CL, Liu TP, Chang KJ, Guo IC (2007) Leptin induces proliferation and anti-apoptosis in human hepatocarcinoma cells by up-regulating cyclin D1 and down-regulating Bax via a Janus kinase 2-linked pathway. Endocr Relat Cancer 14 (2):513–529
54. Saxena NK, Sharma D, Ding X, Lin S, Marra F, Merlin D, Anania FA (2007) Concomitant activation of the JAK/STAT, PI3K/AKT, and ERK signaling is involved in leptin-mediated promotion of invasion and migration of hepatocellular carcinoma cells. Cancer Res 67(6):2497–2507
55. Ikejima K, Takei Y, Honda H, Hirose M, Yoshikawa M, Zhang YJ, Lang T, Fukuda T, Yamashina S, Kitamura T, Sato N (2002) Leptin receptor-mediated signaling regulates hepatic fibrogenesis and remodeling of extracellular matrix in the rat. Gastroenterology 122 (5):1399–1410
56. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y (2000) Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 20(6):1595–1599
57. Kamada Y, Matsumoto H, Tamura S, Fukushima J, Kiso S, Fukui K, Igura T, Maeda N, Kihara S, Funahashi T, Matsuzawa Y, Shimomura I, Hayashi N (2007) Hypoadiponectinemia accelerates hepatic tumor formation in a nonalcoholic steatohepatitis mouse model. J Hepatol 47(4):556–564
58. Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ (2003) The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. J Clin Invest 112 (1):91–100
59. Saxena NK, Fu PP, Nagalingam A, Wang J, Handy J, Cohen C, Tighiouart M, Sharma D, Anania FA (2010) Adiponectin modulates C-jun N-terminal kinase and mammalian target of rapamycin and inhibits hepatocellular carcinoma. Gastroenterology 139 (5):1762–1773, 1773 e1761-1765
60. Shimizu M, Yasuda Y, Sakai H, Kubota M, Terakura D, Baba A, Ohno T, Kochi T, Tsurumi H, Tanaka T, Moriwaki H (2011) Pitavastatin suppresses diethylnitrosamine-induced liver preneoplasms in male C57BL/KsJ-db/db obese mice. BMC Cancer 11(1):281
61. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr (2003) Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112(12):1796–1808
62. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 112(12):1821–1830
63. Hotamisligil GS, Shargill NS, Spiegelman BM (1993) Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259(5091):87–91
64. Hotamisligil GS, Budavari A, Murray D, Spiegelman BM (1994) Reduced tyrosine kinase activity of the insulin receptor in obesity–diabetes. Central role of tumor necrosis factor-alpha. J Clin Invest 94(4):1543–1549
65. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS (1997) Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. Nature 389(6651):610–614
66. Park EJ, Lee JH, Yu GY, He G, Ali SR, Holzer RG, Osterreicher CH, Takahashi H, Karin M (2010) Dietary and genetic obesity

promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. Cell 140(2):197–208
67. Senn JJ, Klover PJ, Nowak IA, Mooney RA (2002) Interleukin-6 induces cellular insulin resistance in hepatocytes. Diabetes 51 (12):3391–3399
68. Pickup JC, Mattock MB, Chusney GD, Burt D (1997) NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. Diabetologia 40(11):1286–1292
69. Donath MY, Shoelson SE (2011) Type 2 diabetes as an inflammatory disease. Nat Rev Immunol 11(2):98–107
70. Nov O, Kohl A, Lewis EC, Bashan N, Dvir I, Ben-Shlomo S, Fishman S, Wueest S, Konrad D, Rudich A (2010) Interleukin-1beta may mediate insulin resistance in liver-derived cells in response to adipocyte inflammation. Endocrinology 151 (9):4247–4256
71. Spranger J, Kroke A, Mohlig M, Hoffmann K, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF (2003) Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes 52(3):812–817
72. Faggioni R, Feingold KR, Grunfeld C (2001) Leptin regulation of the immune response and the immunodeficiency of malnutrition. FASEB J 15(14):2565–2571
73. Molina A, Vendrell J, Gutierrez C, Simon I, Masdevall C, Soler J, Gomez JM (2003) Insulin resistance, leptin and TNF-alpha system in morbidly obese women after gastric bypass. Obes Surg 13 (4):615–621
74. Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, Shoelson SE (2005) Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nat Med 11(2):183–190
75. Solinas G, Karin M (2010) JNK1 and IKKbeta: molecular links between obesity and metabolic dysfunction. FASEB J 24 (8):2596–2611
76. Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS (2002) A central role for JNK in obesity and insulin resistance. Nature 420(6913):333–336
77. Shoelson SE, Lee J, Goldfine AB (2006) Inflammation and insulin resistance. J Clin Invest 116(7):1793–1801
78. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I (2004) Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 114(12):1752–1761
79. Solinas G, Naugler W, Galimi F, Lee MS, Karin M (2006) Saturated fatty acids inhibit induction of insulin gene transcription by JNK-mediated phosphorylation of insulin-receptor substrates. Proc Natl Acad Sci U S A 103(44):16454–16459
80. Lee JY, Sohn KH, Rhee SH, Hwang D (2001) Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4. J Biol Chem 276(20):16683–16689
81. Malhi H, Barreyro FJ, Isomoto H, Bronk SF, Gores GJ (2007) Free fatty acids sensitise hepatocytes to TRAIL mediated cytotoxicity. Gut 56(8):1124–1131
82. Feldstein AE, Werneburg NW, Canbay A, Guicciardi ME, Bronk SF, Rydzewski R, Burgart LJ, Gores GJ (2004) Free fatty acids promote hepatic lipotoxicity by stimulating TNF-alpha expression via a lysosomal pathway. Hepatology 40(1):185–194
83. Malhi H, Gores GJ (2008) Molecular mechanisms of lipotoxicity in nonalcoholic fatty liver disease. Semin Liver Dis 28(4):360–369
84. Schattenberg JM, Singh R, Wang Y, Lefkowitch JH, Rigoli RM, Scherer PE, Czaja MJ (2006) JNK1 but not JNK2 promotes the development of steatohepatitis in mice. Hepatology 43(1):163–172
85. Wang Y, Ausman LM, Greenberg AS, Russell RM, Wang XD (2009) Nonalcoholic steatohepatitis induced by a high-fat diet promotes diethylnitrosamine-initiated early hepatocarcinogenesis in rats. Int J Cancer 124(3):540–546
86. Shimizu M, Sakai H, Shirakami Y, Iwasa J, Yasuda Y, Kubota M, Takai K, Tsurumi H, Tanaka T, Moriwaki H (2011) Acyclic retinoid inhibits diethylnitrosamine-induced liver tumorigenesis in obese and diabetic C57BLKS/J-+(db)/+Lepr(db) mice. Cancer Prev Res 4(1):128–136
87. He G, Yu GY, Temkin V, Ogata H, Kuntzen C, Sakurai T, Sieghart W, Peck-Radosavljevic M, Leffert HL, Karin M (2010) Hepatocyte IKKbeta/NF-kappaB inhibits tumor promotion and progression by preventing oxidative stress-driven STAT3 activation. Cancer Cell 17(3):286–297
88. Giovannucci E, Michaud D (2007) The role of obesity and related metabolic disturbances in cancers of the colon, prostate, and pancreas. Gastroenterology 132(6):2208–2225
89. Frezza EE, Wachtel MS, Chiriva-Internati M (2006) Influence of obesity on the risk of developing colon cancer. Gut 55(2):285–291
90. Hirose Y, Hata K, Kuno T, Yoshida K, Sakata K, Yamada Y, Tanaka T, Reddy BS, Mori H (2004) Enhancement of development of azoxymethane-induced colonic premalignant lesions in C57BL/KsJ-db/db mice. Carcinogenesis 25(5):821–825
91. Shimizu M, Shirakami Y, Iwasa J, Shiraki M, Yasuda Y, Hata K, Hirose Y, Tsurumi H, Tanaka T, Moriwaki H (2009) Supplementation with branched-chain amino acids inhibits azoxymethane-induced colonic preneoplastic lesions in male C57BL/KsJ-db/db mice. Clin Cancer Res 15(9):3068–3075
92. Shimizu M, Shirakami Y, Sakai H, Adachi S, Hata K, Hirose Y, Tsurumi H, Tanaka T, Moriwaki H (2008) (−)-Epigallocatechin gallate suppresses azoxymethane-induced colonic premalignant lesions in male C57BL/KsJ-db/db mice. Cancer Prev Res 1 (4):298–304
93. Moriwaki H, Miwa Y, Tajika M, Kato M, Fukushima H, Shiraki M (2004) Branched-chain amino acids as a protein- and energy-source in liver cirrhosis. Biochem Biophys Res Commun 313 (2):405–409
94. Kawaguchi T, Izumi N, Charlton MR, Sata M (2011) Branched-chain amino acids as pharmacological nutrients in chronic liver disease. Hepatology 54(3):1063–1070
95. Muto Y, Sato S, Watanabe A, Moriwaki H, Suzuki K, Kato A, Kato M, Nakamura T, Higuchi K, Nishiguchi S, Kumada H, Long-Term Survival Study Group (2005) Effects of oral branched-chain amino acid granules on event-free survival in patients with liver cirrhosis. Clin Gastroenterol Hepatol 3 (7):705–713
96. Marchesini G, Bianchi G, Merli M, Amodio P, Panella C, Loguercio C, Rossi Fanelli F, Abbiati R (2003) Nutritional supplementation with branched-chain amino acids in advanced cirrhosis: a double-blind, randomized trial. Gastroenterology 124 (7):1792–1801
97. Kao YH, Chang HH, Lee MJ, Chen CL (2006) Tea, obesity, and diabetes. Mol Nutr Food Res 50(2):188–210
98. Hursel R, Viechtbauer W, Westerterp-Plantenga MS (2009) The effects of green tea on weight loss and weight maintenance: a meta-analysis. Int J Obes 33(9):956–961
99. Yang CS, Wang X, Lu G, Picinich SC (2009) Cancer prevention by tea: animal studies, molecular mechanisms and human relevance. Nat Rev Cancer 9(6):429–439
100. Shimizu M, Adachi S, Masuda M, Kozawa O, Moriwaki H (2011) Cancer chemoprevention with green tea catechins by targeting receptor tyrosine kinases. Mol Nutr Food Res 55 (6):832–843
101. Muto Y, Moriwaki H, Ninomiya M, Adachi S, Saito A, Takasaki KT, Tanaka T, Tsurumi K, Okuno M, Tomita E, Nakamura T,

Kojima T (1996) Prevention of second primary tumors by an acyclic retinoid, polyprenoic acid, in patients with hepatocellular carcinoma. Hepatoma Prevention Study Group. N Engl J Med 334(24):1561–1567
102. Muto Y, Moriwaki H, Saito A (1999) Prevention of second primary tumors by an acyclic retinoid in patients with hepatocellular carcinoma. N Engl J Med 340(13):1046–1047
103. Shimizu M, Takai K, Moriwaki H (2009) Strategy and mechanism for the prevention of hepatocellular carcinoma: phosphorylated retinoid X receptor alpha is a critical target for hepatocellular carcinoma chemoprevention. Cancer Sci 100 (3):369–374
104. Shimizu M, Sakai H, Moriwaki H (2011) Chemoprevention of hepatocellular carcinoma by acyclic retinoid. Front Biosci 16:759–769
105. Petrides AS, Vogt C, Schulze-Berge D, Matthews D, Strohmeyer G (1994) Pathogenesis of glucose intolerance and diabetes mellitus in cirrhosis. Hepatology 19(3):616–627
106. Mehta SH, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL (2000) Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. Ann Intern Med 133(8):592–599
107. She P, Reid TM, Bronson SK, Vary TC, Hajnal A, Lynch CJ, Hutson SM (2007) Disruption of BCATm in mice leads to increased energy expenditure associated with the activation of a futile protein turnover cycle. Cell Metab 6(3):181–194
108. Higuchi N, Kato M, Miyazaki M, Tanaka M, Kohjima M, Ito T, Nakamuta M, Enjoji M, Kotoh K, Takayanagi R (2011) Potential role of branched-chain amino acids in glucose metabolism through the accelerated induction of the glucose-sensing apparatus in the liver. J Cell Biochem 112(1):30–38
109. Kawaguchi T, Nagao Y, Matsuoka H, Ide T, Sata M (2008) Branched-chain amino acid-enriched supplementation improves insulin resistance in patients with chronic liver disease. Int J Mol Med 22(1):105–112
110. Urata Y, Okita K, Korenaga K, Uchida K, Yamasaki T, Sakaida I (2007) The effect of supplementation with branched-chain amino acids in patients with liver cirrhosis. Hepatol Res 37(7):510–516
111. Hagiwara A, Nishiyama M, Ishizaki S (2012) Branched-chain amino acids prevent insulin-induced hepatic tumor cell proliferation by inducing apoptosis through mTORC1 and mTORC2-dependent mechanisms. J Cell Physiol 227(5):2097–2105
112. Yoshiji H, Noguchi R, Kitade M, Kaji K, Ikenaka Y, Namisaki T, Yoshii J, Yanase K, Yamazaki M, Tsujimoto T, Akahane T, Kawaratani H, Uemura M, Fukui H (2009) Branched-chain amino acids suppress insulin-resistance-based hepatocarcinogenesis in obese diabetic rats. J Gastroenterol 44(5):483–491
113. Qin B, Polansky MM, Harry D, Anderson RA (2010) Green tea polyphenols improve cardiac muscle mRNA and protein levels of signal pathways related to insulin and lipid metabolism and inflammation in insulin-resistant rats. Mol Nutr Food Res 54 (Suppl 1):S14–S23
114. Bose M, Lambert JD, Ju J, Reuhl KR, Shapses SA, Yang CS (2008) The major green tea polyphenol, (−)-epigallocatechin-3-gallate, inhibits obesity, metabolic syndrome, and fatty liver disease in high-fat-fed mice. J Nutr 138(9):1677–1683
115. Ramadan G, El-Beih NM, Abd El-Ghffar E (2009) Modulatory effects of black v. green tea aqueous extract on hyperglycaemia, hyperlipidaemia and liver dysfunction in diabetic and obese rat models. Br J Nutr 102(11):1611–1619
116. Shirakami Y, Shimizu M, Tsurumi H, Hara Y, Tanaka T, Moriwaki H (2008) EGCG and Polyphenon E attenuate inflammation-related mouse colon carcinogenesis induced by AOM plus DDS. Mol Med Report 1(3):355–361
117. Shimizu M, Deguchi A, Hara Y, Moriwaki H, Weinstein IB (2005) EGCG inhibits activation of the insulin-like growth factor-1 receptor in human colon cancer cells. Biochem Biophys Res Commun 334(3):947–953
118. Yasuda Y, Shimizu M, Sakai H, Iwasa J, Kubota M, Adachi S, Osawa Y, Tsurumi H, Hara Y, Moriwaki H (2009) (−)-Epigallocatechin gallate prevents carbon tetrachloride-induced rat hepatic fibrosis by inhibiting the expression of the PDGFRbeta and IGF-1R. Chem Biol Interact 182(2–3):159–164
119. Shimizu M, Fukutomi Y, Ninomiya M, Nagura K, Kato T, Araki H, Suganuma M, Fujiki H, Moriwaki H (2008) Green tea extracts for the prevention of metachronous colorectal adenomas: a pilot study. Cancer Epidemiol Biomarkers Prev 17(11):3020–3025
120. Bettuzzi S, Brausi M, Rizzi F, Castagnetti G, Peracchia G, Corti A (2006) Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study. Cancer Res 66(2):1234–1240
121. Li N, Sun Z, Han C, Chen J (1999) The chemopreventive effects of tea on human oral precancerous mucosa lesions. Proc Soc Exp Biol Med 220(4):218–224
122. Germain P, Chambon P, Eichele G, Evans RM, Lazar MA, Leid M, De Lera AR, Lotan R, Mangelsdorf DJ, Gronemeyer H (2006) International Union of Pharmacology. LXIII. Retinoid X receptors. Pharmacol Rev 58(4):760–772
123. Germain P, Chambon P, Eichele G, Evans RM, Lazar MA, Leid M, De Lera AR, Lotan R, Mangelsdorf DJ, Gronemeyer H (2006) International Union of Pharmacology. LX. Retinoic acid receptors. Pharmacol Rev 58(4):712–725
124. Altucci L, Gronemeyer H (2001) The promise of retinoids to fight against cancer. Nat Rev Cancer 1(3):181–193
125. Altucci L, Leibowitz MD, Ogilvie KM, de Lera AR, Gronemeyer H (2007) RAR and RXR modulation in cancer and metabolic disease. Nat Rev Drug Discov 6(10):793–810
126. Ando N, Shimizu M, Okuno M, Matsushima-Nishiwaki R, Tsurumi H, Tanaka T, Moriwaki H (2007) Expression of retinoid X receptor alpha is decreased in 3′-methyl-4-dimethylaminoazobenzene-induced hepatocellular carcinoma in rats. Oncol Rep 18 (4):879–884
127. Matsushima-Nishiwaki R, Okuno M, Adachi S, Sano T, Akita K, Moriwaki H, Friedman SL, Kojima S (2001) Phosphorylation of retinoid X receptor alpha at serine 260 impairs its metabolism and function in human hepatocellular carcinoma. Cancer Res 61 (20):7675–7682
128. Matsushima-Nishiwaki R, Okuno M, Takano Y, Kojima S, Friedman SL, Moriwaki H (2003) Molecular mechanism for growth suppression of human hepatocellular carcinoma cells by acyclic retinoid. Carcinogenesis 24(8):1353–1359
129. Adachi S, Okuno M, Matsushima-Nishiwaki R, Takano Y, Kojima S, Friedman SL, Moriwaki H, Okano Y (2002) Phosphorylation of retinoid X receptor suppresses its ubiquitination in human hepatocellular carcinoma. Hepatology 35(2):332–340
130. Yoshimura K, Muto Y, Shimizu M, Matsushima-Nishiwaki R, Okuno M, Takano Y, Tsurumi H, Kojima S, Okano Y, Moriwaki H (2007) Phosphorylated retinoid X receptor alpha loses its heterodimeric activity with retinoic acid receptor beta. Cancer Sci 98(12):1868–1874
131. Kanamori T, Shimizu M, Okuno M, Matsushima-Nishiwaki R, Tsurumi H, Kojima S, Moriwaki H (2007) Synergistic growth inhibition by acyclic retinoid and vitamin K2 in human hepatocellular carcinoma cells. Cancer Sci 98(3):431–437
132. Okita K, Matsui O, Kumada H, Tanaka K, Kaneko S, Moriwaki H, Izumi N, Okusaka T, Ohashi Y, Makuuchi M (2010) Effect of peretinoin on recurrence of hepatocellular carcinoma (HCC): results of a phase II/III randomized placebo-controlled trial. J Clin Oncol 28(Suppl 7s):4024

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REVIEW ARTICLE

Chemical-induced Carcinogenesis

Takuji Tanaka [1,2,3]*, Masahito Shimizu [4], Takahiro Kochi [4], Hisataka Moriwaki [4]

[1] Clin-ToxPath (C-Top) Consulting, Ichihashi, Gifu City, Japan
[2] Tohkai Cytopathology Institute, Cancer Research and Prevention, Minami-Uzura, Gifu, Japan
[3] Department of Tumor Pathology, Gifu University Graduate School of Medicine, Yanagido, Gifu, Japan
[4] Department of Medicine, Gifu University Graduate School of Medicine, Yanagido, Gifu, Japan

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Historically, evidence of chemical carcinogenesis has played a significant role in verifying conclusions draw from epidemiological studies. Chemical agents that were suspected to have a certain role in human chronic diseases, such as cancers, have been tested in animals to establish firmly a causative risk or link to risk. The three best examples are: (1) tobacco smoke and lung cancer; (2) asbestos and mesothelioma; and (3) aflatoxin and hepatic cancer. New chemical compounds are synthesized every day, and a number of natural or synthetic compounds are incorporated in foods either as a result of their processing or to preserve or enhance them. Chemical carcinogenesis studies using model animals have greatly contributed to understanding the mechanisms underlying the development and prevention of carcinogenesis. The carcinogenesis process is generally considered to include three steps: initiation, promotion, and progression. Each step is characterized by morphological and biochemical alterations resulting from genetic and epigenetic changes, including mutations in proto-oncogenes and tumor suppressor genes that control proliferation, cell death, and cellular repair. Long-term *in vivo* assays using laboratory animals enable the identification of carcinogenic compounds and their modes of action. Based on these findings, we should be able to establish effective strategies to treat and prevent malignancies resulting from exposure to potentially carcinogenic chemicals.

1. Introduction

Neoplasms can be classified as benign or malignant depending on their biological characteristics. The malignant cells show a variety of biological features (Figure 1). They proliferate autonomously, invade adjacent tissues, and frequently metastasize to distant tissues that are not related to the primary site.[1] The most important biological characteristic of a malignant neoplasm is its ability to metastasize. By contrast, benign neoplasms grow more slowly, but can compress their adjacent normal tissue.[2] Therefore, the histopathological observation/diagnosis of neoplasms (benign or malignant; and epithelial or nonepithelial origin) is important for understanding the pathogenesis and pathobiology of the neoplasms.[3–5] The histological and cytological changes that occur during tumorigenesis are illustrated in Figure 2. Malignant epithelial cells multiply clonally, escape from apoptosis, and accumulate genetic and/or epigenetic alterations.[6] When malignant neoplasms originate from nonepithelial cells, they are called sarcomas. The escape of malignant cells from apoptosis results in uncontrolled growth of neoplastic cells, and this is a critical point that determines the malignant potential of the cells,[7] and thus apoptosis induction is considered to be one of the mechanisms that can be targeted for cancer chemoprevention.[8]

The term "carcinogenic" is defined as the capacity of a chemical compound to induce the development of cancer in certain tissues under certain conditions.[9,10] A compound is considered to be "carcinogenic" when its administration to laboratory animals produces a statistically significant increase in the incidence of several histological types of neoplasms compared with the control group not exposed to the compound.

The carcinogenic factors that are responsible for cancer development are classified as either exogenous or endogenous.[10] The exogenous factors include agents associated with food preservation and preparation, socio-economic status, lifestyle, ionizing and nonionizing radiation, natural and synthetic chemical compounds, and xenobiotics including *Helicobacter pylori*, Epstein–Barr virus, human T-lymphtropic virus, human papillomavirus, hepatitis B virus, hepatitis C virus, and certain parasites.[11,12] Alcohol consumption, tobacco smoking, and the intake of certain foods contaminated by mycotoxins are also responsible for causing certain types of neoplasms.[12]

Endogenous carcinogenic factors include conditions and agents that cause immune system disruption and subsequent

* Corresponding author. Takuji Tanaka, Clin-ToxPath (C-Top) Consulting, 1-7-9 Ichihashi, Gifu City 500-8381, Japan.
E-mail: T. Tanaka <takutt@toukaisaibou.co.jp>

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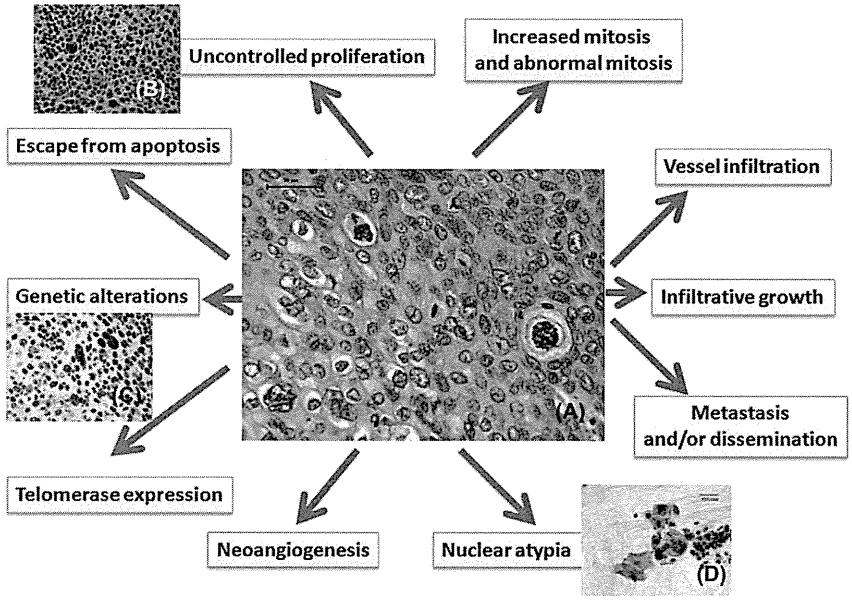

Figure 1 Biological characteristics of malignant cells. (A) Histology of human skin squamous cell carcinoma; (B) PCNA immunohistochemistry; (C) p53 immunohistochemistry, and (D) scraped cytology of human skin cancer. (A) hematoxylin and eosin stain and (D) Papanicolaou stain. Bars are 50 μm (A–C) and 20 μm (D). PCNA = proliferating cell nuclear antigen.

inflammation, such as ulcerative colitis.[2,12–16] Epidemiological studies suggest that the risk of developing cancer varies between different population groups, and these differences are associated with both genetic differences and lifestyle-related factors and habits. Indeed, the migration of certain populations to new regions with different lifestyles can result in the development of new types of cancer not previously prevalent in that group.[17] For example, exposure to Western lifestyles had a substantial impact on breast cancer risk in Asian migrants to the USA during their lifetime.[18] A study conducted by Maskarinec and Noh[19] showed that the migrant effect was strongest for colon and stomach cancers; prostate and breast cancers were affected to a lesser degree; and lung cancer risk differed little between Japanese in Japan and Hawaii. Migration led to lower risk of stomach, esophageal, pancreatic, liver, and cervical cancers, but to higher rates for all other cancers.[19]

Neoplastic development is based on the existence of genetic mutations. In most cases, the effects of such mutations are assumed to vary between tissues and among species. During cell division, spontaneous genetic errors occur with an estimated frequency of around 10^{-5}–10^{-6} nucleotides per cycle of cell division. Although numerous repair systems exist within the cells to correct these errors, if the damage persists and reaches a gene responsible for neoplastic development, then cancer can develop. Indeed, studies

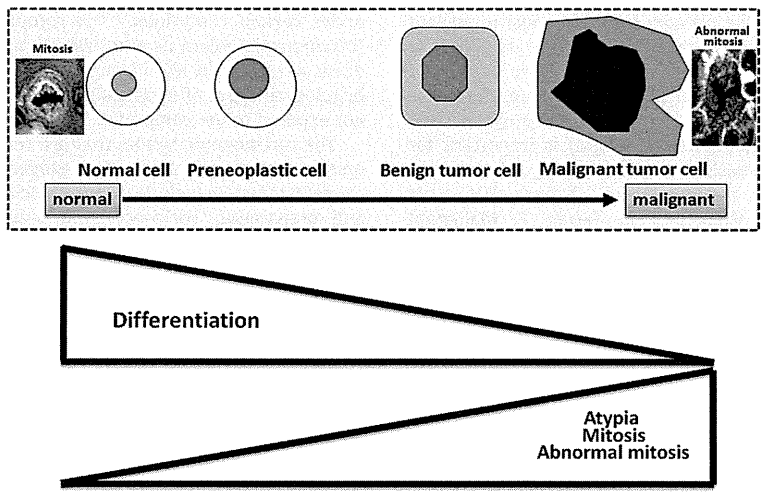

Figure 2 Differentiation and atypia of normal, preneoplastic, and neoplastic cells. Cellular differentiation is decreased during carcinogenesis. Nuclear atypia and number of mitoses including abnormal mitoses are increased during carcinogenesis. An abnormal mitosis in this figure is tripolar mitosis.

to date have consistently shown that human cancer is a genetic disease.[20]

This short review, starting with the historical studies of chemical carcinogenesis, aims to summarize several aspects of chemical carcinogenesis that have been extensively studied to establish causative associations between environmental exposures and increased cancer risk.

2. The history of chemical carcinogenesis

The first experimental work on chemical carcinogenesis was carried out in 1915 by Dr Katsusaburo Yamagiwa (a pathologist) and his assistant Koichi Ichikawa.[21] They painted rabbit ears with coal tar and observed the development of skin squamous cell papillomas and carcinomas. Subsequently, other researchers extensively studied carcinogenesis of other tissues, such as the lungs, bladder, liver, kidneys, and pancreas using laboratory animals, and showed that the experimental use of animals and carcinogens was helpful for studying human cancers, and could provide insight into the causes of cancers.

Drs Beremblum and Shubik used polycyclic aromatic hydrocarbons and croton oil to investigate skin carcinogenesis in mice, and demonstrated that cancer develops through several stages.[22] When applied as a single application to the skin at a low dose, 9,10-dimethyl-1,2-benzanthracene (DMBA) caused only a few or no skin tumors. However, multiple skin tumors developed when croton oil was applied repeatedly after this low-dose DMBA treatment. When croton oil was applied repeatedly prior to the DMBA treatment, no skin tumors developed. Based on these observations, they suggested that carcinogenesis was a complex process that included "initiation" and "promotion" stages. During the next decade, based on the studies by Rous and Beard[23] and Greene,[24] Foulds[25] introduced the term "progression" after investigating experimentally induced breast adenocarcinoma in female mice. Prior to when carcinogens were known to bind to DNA, the cancers produced by chemical carcinogens were believed to be due to their interaction with proteins in specific tissues.[26] By the end of the 1960s, increasing evidence pointed to a correlation between the DNA binding capacity of a carcinogen and its biological potency.[27]

3. Understanding chemical carcinogenesis

3.1. The multiple steps of carcinogenesis

Human cancer development is characterized by the five "Ms", namely multifactorial etiology, multistep, multiyear, multigenetic alterations, and multipath disease. Chemical carcinogenesis also involves multistage and multistep processes. Although the process includes multiple molecular and cellular events that lead to the transformation of normal cells into malignant neoplastic cells, evidence has defined at least three steps in the chemical carcinogenesis process.[3,10] These steps are "initiation",[2] "promotion",[22] and "progression"[25] (Figure 3). The first step, "initiation", is the stage where a normal cell undergoes unrepaired DNA damage and DNA synthesis to produce a mutated (initiated) cell. The production of an initiated cell can occur through interactions with physical carcinogens, i.e., UV light irradiation, as well as chemical carcinogens that possess DNA damaging or mutagenic properties. Additionally, during cell proliferation, mutations may be acquired through misrepair of damaged DNA, resulting in spontaneously initiated (mutated) cells. Following the formation of an initiated cell, chemicals and/or endogenous physiological substances can cause the selective clonal growth of the initiated cell through the process of tumor promotion. Tumor promotion involves the expansion of the initiated cell(s) to a focal lesion. The tumor promotion process is not a direct DNA-reactive or damaging process, but involves modulation of the gene expression, which results in an increase in cell number through cell division and/or decrease in apoptotic cell death.[28] Following continual cell proliferation, additional mutations might be acquired in the preneoplastic cells, resulting in the induction of a neoplasm. The term "conversion" during progression stage implies that benign tumors gain malignant phenotypes. The third step, "progression", involves additional damage to the genome and, unlike the "promotion" step, is irreversible. The multistep

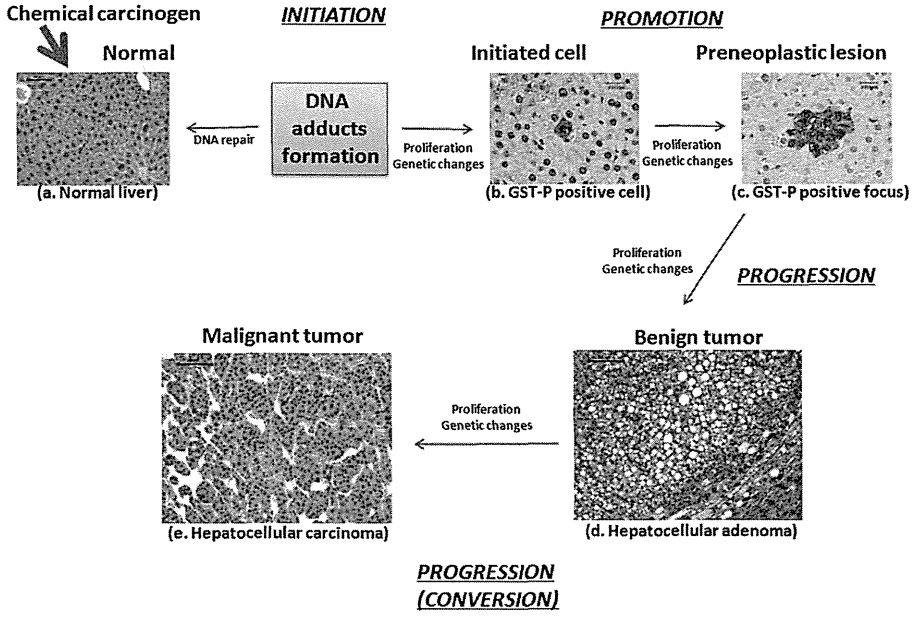

Figure 3 Multistep chemical carcinogenesis.

process has been well defined in rodent systems, and evidence has shown that similar processes occur in humans.

In humans, the clinical detection of a tumor that has developed may not occur for 20–50 years after an individual is exposed to a carcinogen.[29] The multistep process of carcinogenesis has been studied extensively in colon cancer, with the progression from hyperplastic crypts, to adenoma to cancer, and then finally metastasis, all being well characterized.[2]

3.1.1. Initiation

DNA damage can be repaired by enzymatic mechanisms.[30] However, initiated cells that are proliferating have less time to repair damaged DNA and remove covalent bonds with their DNA (DNA adducts).[31] When the initiated cells survive without repair for weeks, months, or years, they can grow in an autonomous and clonal fashion.[32] During the initiation process, cell division remains symmetrical by creating two new initiated cells. Mitogenic stimulation (which leads to an increase in the number of new cells and apoptosis inhibition) by intrinsic and/or extrinsic factors results in the clonal expansion of initiated cells, which then survive. An increase in DNA damage is especially important in stem cells, because damaged stem cells can survive for a long time in the tissues, and may remain hidden.[9]

3.1.2. Promotion

The most important activity of tumor promoters is mitogenic stimulation.[11] In order to exert the tumor-promoting effects that depend on the concentration, the tumor promoter's stimulation must continue for a long duration (weeks, months, or years) in the target tissues.[33] Promotional effects are reversible. When the tumor promoter disappears, regression of the tumor occurs, possibly through apoptosis mechanisms. Some tumor promoters are tissue-specific, but others act simultaneously on several different tissues.[34]

A long-term and/or high-dose exposure, a tumor promoter can sometimes induce preneoplasms and neoplasms even without initiation stimuli.[11] Examples of agents that can cause such lesions are phenobarbital, benzene, asbestos, and arsenic.[6] This is explained by two possibilities: the genotoxicity of these compounds may not be detected, leading to a lack of repair, or the initiated cells may spontaneously develop in response to the insult. In the latter case, an increase in the frequency of cell division can enhance the DNA replication errors as well as mutations. Not all cells exposed to a tumor promoter undergo to the promotion step, and only cells that are stimulated to divide and escape from apoptosis go on to the next step, "progression".[6]

3.1.3. Progression

The sequence of lesions identified by histopathological examinations between the initiation and promotion steps are designated as preneoplasms and/or benign neoplasms.[2,4,5] Their transformation into malignant lesions (with metastasis) is the last step, called "conversion", of the carcinogenesis process.[9,35] During the progression step, a neoplastic or malignant phenotype is obtained through genetic and epigenetic mechanisms.[1,2] In this step, the proliferation is independent of the presence or absence of progression-related stimuli.[36] Progression is characterized by irreversibility, genetic instability, growth factor production, invasion, metastasis, and alterations in the biochemistry, metabolism, and morphology of affected cells.[11,37] Neoangiogenesis is essential to the neoplastic progression.

3.1.4. Metabolism of chemical carcinogens

The metabolism of carcinogens has been discussed mainly in terms of the enzymes involved in the activation[38] and detoxification[39] of these chemicals. Miller[40] and Ames et al[41] developed the concepts of bioactivation, detoxification, and genotoxicity of carcinogens. Chemical carcinogens are absorbed after their oral, inhaled, cutaneous, or injection-based exposure, and are distributed in a variety of tissues.[42] The substances absorbed orally pass through the liver, and only then are they distributed to the other tissues. The carcinogens that first enter the lungs following inhalation are distributed by the bloodstream prior to reaching the liver.[43] The carcinogens that act directly on DNA are classified as direct-acting carcinogens. However, most chemicals require enzymatic conversion to act as carcinogens, and thus it is often the metabolites of compounds that cause the neoplastic changes (Figure 4). These carcinogens are classified as indirect-acting carcinogens or procarcinogens.[44] Metabolic activation, mostly in the liver, is controlled by Phase I reactions, whereas Phase II reactions generally protect the tissues through the transformation of activated compounds into inert products that are easily eliminated from the body.[35,45]

Metabolic activation occurs predominantly in the liver at the plain endoplasmic reticulum where the cytochrome P450s are abundant, and to a lesser degree in other tissues, including the bladder, skin, gastrointestinal tract, esophagus, kidneys, and lungs. During Phase I reactions, the cytochrome P450 monooxygenases introduce a reactive polar group into the carcinogen, making it lipophilic, and then convert it into a powerful electrophilic product that is capable of causing DNA adduct formation.[46] Phase II reactions are catalyzed by hepatic and extra-hepatic, cytoplasmic and cytochromic enzymes, acting separately or cooperatively.[47] Conjugation reactions enable these enzymes to decompose the polar group in glucose, amino acids, glutathione, and sulfate, which are less toxic metabolites that are more soluble in water and more easily excreted via the urine and bile.[48]

The metabolic activation of carcinogens is equally important for both humans and animals, although there are qualitative and quantitative differences between them, leading to incorrect interpretations when animal models are used in the research and analysis of the carcinogenic properties of chemical compounds.[49] There are several exogenous and endogenous factors that influence the susceptibility to carcinogenesis.[50]

3.1.5. Epigenetic mechanisms involved in chemical carcinogenesis

The most well understood epigenetic mechanisms involve DNA methylation and histone acetylation, methylation, and phosphorylation. The demethylation of promoter regions at the CpG sequences can lead to an overexpression of proto-oncogenes, and silencing of gene expression can occur as a result of hypermethylation, sometimes leading to chromosome condensation.[35] There appears to be a relationship between DNA methylation and histone modifications; patterns of histone deacetylation and histone methylation are associated with DNA methylation and gene silencing. Interestingly, these epigenetic changes in chromatin can also alter the sensitivity of DNA sequences to mutation, thus rendering genes more or less susceptible to a toxic insult.[37]

4. Molecular targets of chemical carcinogens

When oncogenes are transfected into immortalized mouse cell lines, they are able to induce neoplastic transformation. However, there are other genes that can influence neoplastic transformation.[30] For example, there are several genes that intervene in carcinogenesis.[30,51] Alterations in proto-oncogenes, tumor suppressor genes, and cell cycle regulatory genes are especially important during carcinogenesis.[7,35,52] Although there are several genetic diseases where mutations in one gene can cause disease, neoplastic development requires the presence of errors in the cellular defense mechanisms, which are controlled by checkpoints