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Retinoids Decrease Soluble MICA Concentration by Inhibiting the Enzymatic Activity of ADAM9 and ADAM10

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Abstract. *Background/Aim: The association between MHC class I polypeptide-related sequence A (MICA) and hepatocellular carcinoma (HCC) development was identified in our previous genome-wide association study. Decreasing soluble MICA (sMICA) through MICA sheddases suppression facilitates natural killer (NK) cell-mediated cytotoxicity. The expression of ADAM9 in HCC has been correlated with poor prognosis, and our recent study showed that its suppression contributes to cancer elimination by decreasing sMICA. Materials and Methods: Human HCC cell line PLC/PRF/5 and HepG2 cells were used. sMICA levels were measured by ELISA. Expression of retinoid X receptors (RXRs) and retinoic acid receptors (RARs) was knocked down by siRNA. Results: In our screening of FDA-approved drugs in vitro, retinoids were found to be efficient ADAM9 and ADAM10 inhibitors. Treatment with retinoids reduced sMICA levels in human HCC cells. Interestingly, the effects were abrogated by depletion of the retinoid receptor RXRα. Conclusion: Retinoids can be potential novel agents for HCC treatment.*

Even with the recent advancements in clinical management, patients with chronic fibrotic liver disease due to viral or metabolic etiologies are at a high risk of developing hepatocellular carcinoma (HCC) (1). Chronic hepatitis B or C infection is a major cause of HCC in Japan (2). HCC is a common disease refractory to therapeutic interventions because 70% of patients experience tumor recurrence within five years even after surgical resection or radiofrequency ablation (3). Therefore, effective cancer prevention therapy is highly required for improving their prognosis.

Peretinoin, an acyclic retinoid and vitamin A analogue, significantly reduces the incidence of second primary hepatomas (4). Since retinoids mainly inhibit the promotion phase but not the advanced phases (*i.e.*, conversion and progression) of carcinogenesis, are considered as chemopreventive rather than chemotherapeutic medicine (5).

Peretinoin also inhibits multiple cellular signaling pathways as exemplified by Wnt and platelet derived growth factor, thereby inducing differentiation and apoptosis of hepatic stem cells and neoplasm suppression (6, 7). In terms of tumor microenvironment, the function of innate immunity is crucial to eliminate neoplastic clones and prevent spreading of recurring or metastatic cancers (8). However, the precise mechanisms of its effects on innate immunity in HCC have not yet been elucidated.

In our previous genome-wide association study (GWAS) related to HCC development, MHC class I polypeptide-related sequence A (MICA) was identified as a susceptibility gene (9). MICA, which is not expressed on normal hepatocytes but rather on virus-infected hepatocytes or HCC cells, works as a natural killer group D (NKG2D) ligand to elicit a direct attack by natural killer (NK) cells (9). The membrane-bound MICA (mMICA) is released as soluble MICA (sMICA) into patients' serum, which acts as an immunological decoy for cancer immunity prevention by NK cells. Our previous research indicated that similar to ADAM9 and ADAM10, ADAM17 contributed to the release of sMICA (10). Hu *et al*. have demonstrated the relationship

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Key Words: HCC treatment, retinoids, ADAM9, ADAM10, MICA.

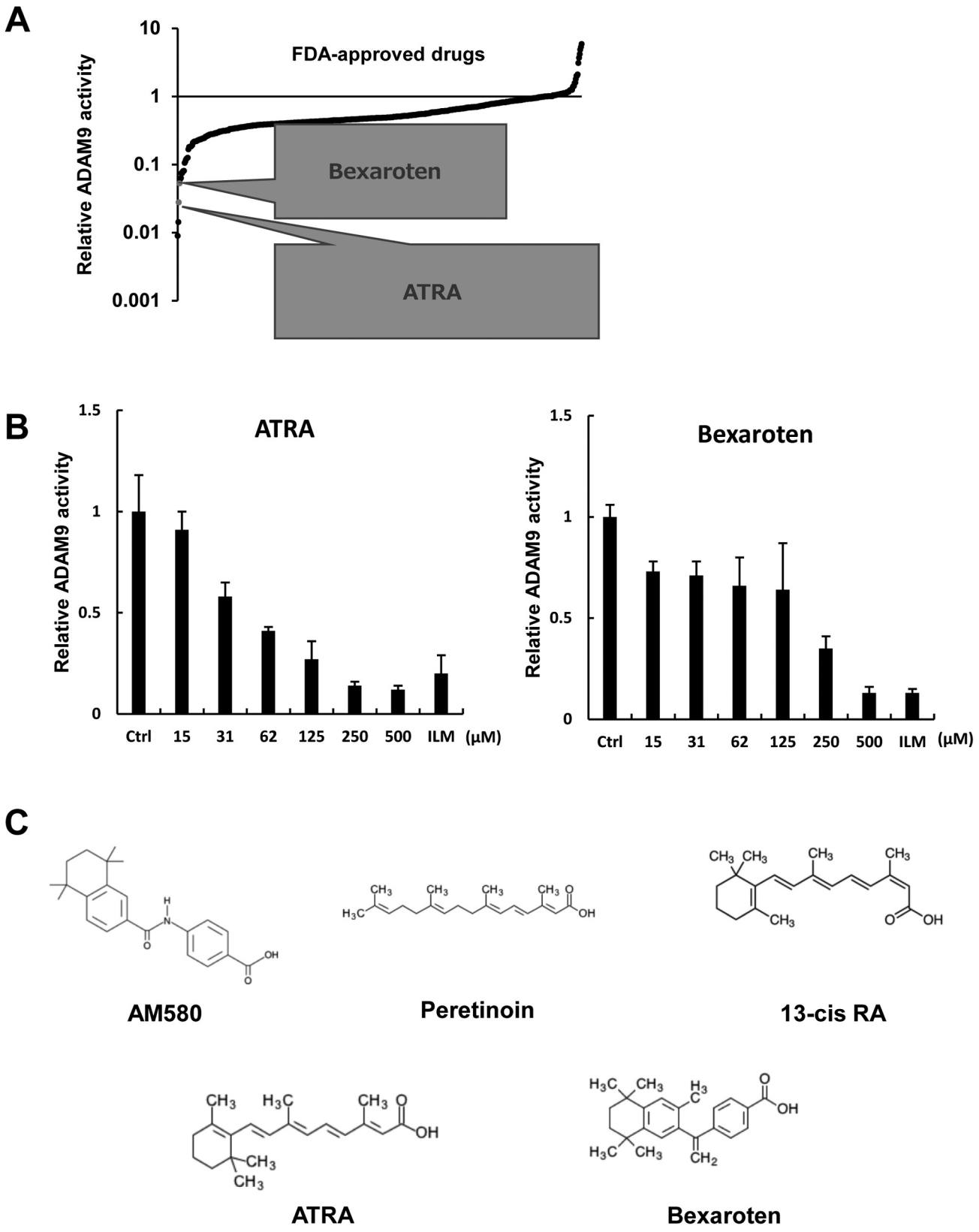
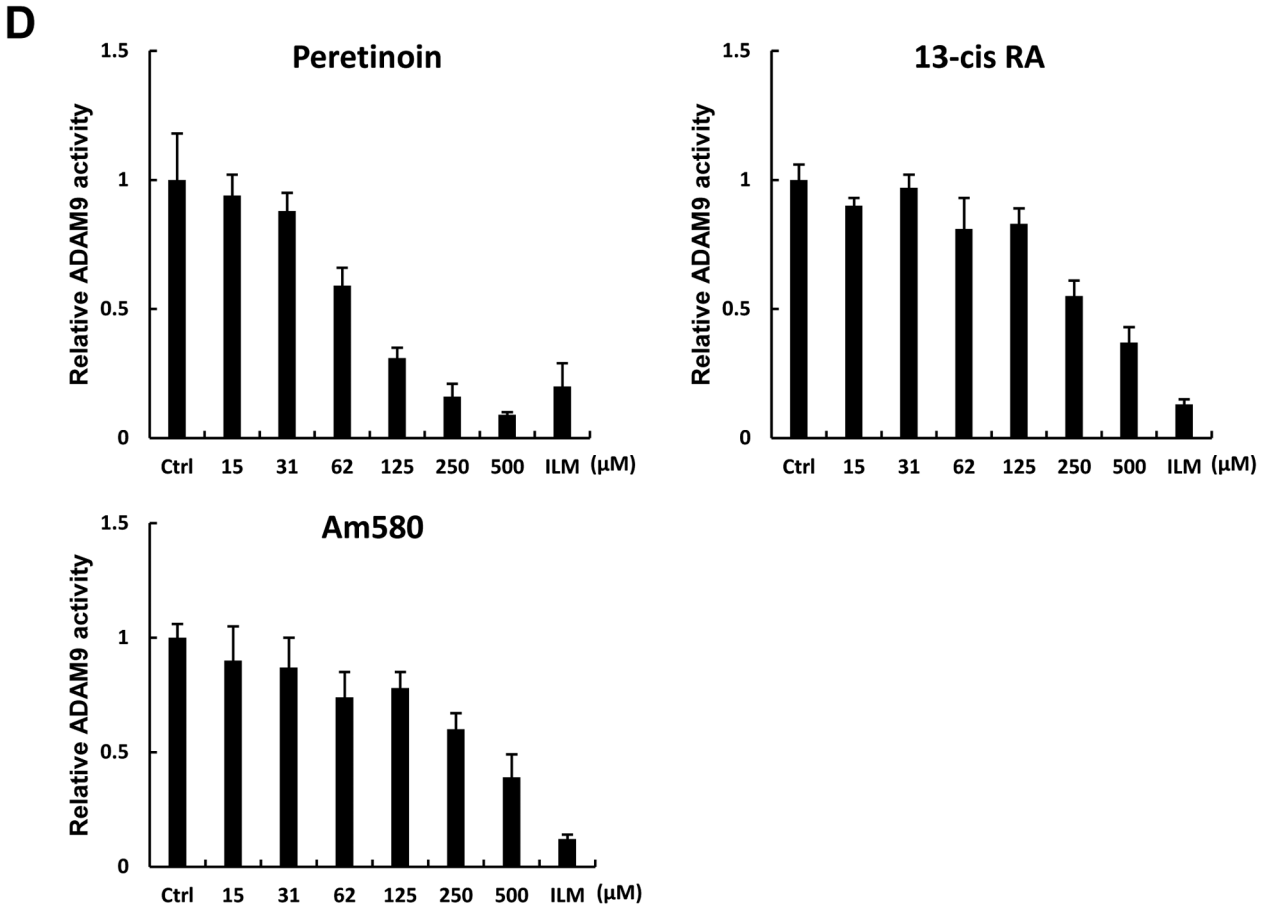


Figure 1. *Continued*



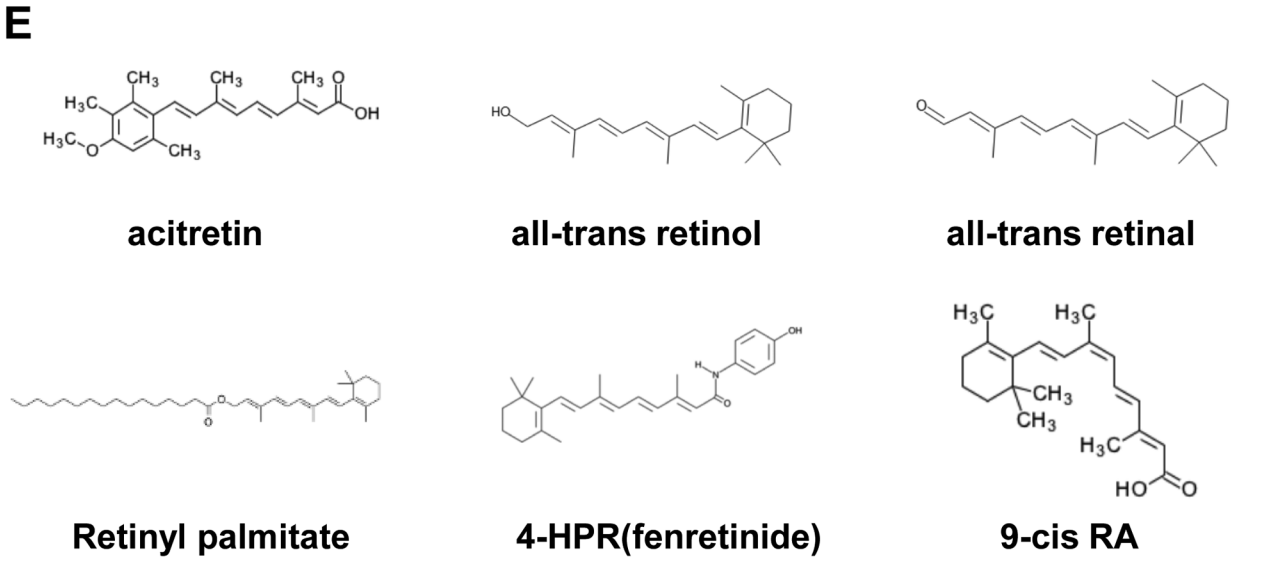


Figure 1. *Retinoids inhibited ADAM9 in vitro. (A) Inhibitory effects of approved drugs on ADAM9 in vitro. (B) Dose-dependent inhibition of ADAM9 by ATRA and bexaroten in vitro. (C) Molecular structures of retinoids. (D) Dose-dependent inhibition of ADAM9 by peretinoin, 13-cis RA, and Am580 in vitro. Ilomastat (ILM) is used as the ADAM9 control inhibitor. Error bars represent standard error of the mean (SEM). The results derive from three independent experiments. (E) Molecular structures of other retinoids.*

between ADAM9 expression and clinicopathological features, which include disease prognosis, short overall survival, tumor grade, metastasis, and the development of resistance in various cancers including HCC (11).

Therefore, we recently established a new *in vitro* assay to evaluate the enzymatic activity of ADAM9 and a screen using a library of FDA-approved drugs identified that leukotriene C4 and leukotriene D4 suppression using leukotriene receptor antagonists (LTRAs) could enhance NK cell-mediated elimination of cancer cells through ADAM9 inhibition and subsequent decrease in MICA shedding (12). Our previous study has also clarified that multi-kinase inhibitor regorafenib (REG) decreased sMICA concentration by mainly targeting ADAM9 transcriptionally, not enzymatically (13).

Here, we evaluated the influence of another group of ADAM9 enzyme inhibitors from the FDA-approved drug library on sMICA release in HCC cells. In addition, the effect of the combination treatment with REG on the sMICA levels was also analyzed.

Materials and Methods

Cells, reagents, and antibodies. The FDA-Approved Drug Screen-well library was obtained from Enzo Life Sciences (Farmingdale, NY, USA). HepG2 and PLC/PRF/5 cells were obtained from American Type Culture Collection (Manassas, VA, USA) and cultured according to the manufacturer's protocols. The cell lines were authenticated by short tandem repeat analysis (Bex, Tokyo, Japan) in November 2020. All-trans retinoic acid (ATRA) and all-trans retinal were obtained from FUJIFILM WAKO Pure Chemical corporation (Osaka, Japan). 13-cis retinoic acid (RA), acitretin, and bexarotene were obtained from TCI (Tokyo, Japan). 11-cis RA and 9,13-di-cis RA were purchased from Santa Cruz Biotechnology (Dallas, TX, USA).

Am580, peretinoin, 9-cis RA, all-trans retinol, 4-HPR, and retinyl palmitate, were obtained from Cayman chemical (Ann Arbor, MC, USA), Medchemexpress (Monmouth, NJ, USA), LKT Labs (St.Paul, MN, USA), CEM (Matthews, NC, USA), and ChromaDex (Los Angeles, CA, USA), respectively.

Cell Counting Kit-8 (CCK8), and regorafenib were purchased from Dojindo (Kumamoto, Japan) and Cell Signaling Technology (Danvers, MA, USA), respectively.

The siRNA for ADAM9, RXRα, RXRβ, RXRγ, RARα, RARβ, RARγ, and the control siRNA were purchased from Dharmacon (Ann Arbor, MI, USA) and Cell Signaling Technology, respectively.

Cell viability assays. HepG2 and PLC/PRF/5 cells (2×10^5 cells/ml/well) were plated in 24-well plates and incubated at 37˚C for 24 h. The cells were treated with retinoids, or regorafenib for 48 h. After the treatment, the culture supernatant was collected, and cell viability was measured using the CCK8 assay kit (Dojindo). Briefly, 1 ml CCK-8 reagent diluted following the manufacturer's instructions was added per well and the plates were incubated at 37˚C for 1 h. After incubation, absorbance at 450 nm was measured using a microplate reader (xMark™ Microplate spectrophotometer, BIO-RAD Laboratories, Tokyo, Japan) to determine the number of viable cells.

Enzyme linked immunosorbent assay (ELISA). The concentration of sMICA in HepG2 and PLC/PRF/5 cell culture supernatants was assessed using a MICA ELISA Kit (Diaclone, Besançon, France) as described previously (13).

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Relative mRNA levels were quantified as previously described (13) using the following primer sets:
MICA-F: 5'-CTTCCTGCTTCTGGCTGGCATC-3',
MICA-R: 5'-CAGGGTCATCCTGAGGTCCTTTC-3',
ADAM9-F: 5'-AAGAATTGTCACTGTGAAAATGGCT-3',
ADAM9-R: 5'-CATTGTATGTAGGTCCACTGTCCAC-3',
ADAM10-F: 5'-ACGGAACACGAGAAGCTGTG-3',
ADAM10-R: 5'-CCGGAGAAGTCTGTGGTCTG-3',
ADAM17-F: 5'-GTCGAGCCTGGCGGTAGAATCTTC-3',
ADAM17-R: 5'-CTCCACCTCTCTGGGCAGCCTTC-3',
GAPDH-F: 5'-ATGGGGAAGGTGAAGGTCG-3',
GAPDH-R: 5'-GGGGTCATTGATGGCAACAATA-3'.

In vitro ADAM assay. Fifty μl of recombinant human ADAM9 (R&D systems, Minneapolis, MN, USA); 2 μg/ml diluted with assay buffer) was incubated with 50 μl of fluorescent peptide substrate (BioZyme, Apex, NC, USA; 20 μM diluted with assay buffer) in the presence of 1 μl of DMSO or individual compounds, following the manufacturer's instructions. A library of FDA-approved drugs was tested for enzymatic inhibition of ADAM9, and ilomastat was used as the ADAM9 control inhibitor. After incubating for 24 h at 37˚C in opaque black plates, the fluorescent signals (λ excitation=485 nm, λ emission=530 nm) were measured and relative enzymatic activities were calculated. A similar assay was performed with recombinant human ADAM10 and ADAM17 (R&D systems; 2 μg/ml), in which marimastat and TMI-1 were used as the ADAM10 and ADAM17 control inhibitors, respectively.

Knockdown of RXRs and RARs with siRNA. For the specific knockdown of RXRs and RARs, the ON-TARGETplus SMARTpool (Dharmacon) siRNA duplex mixtures were used as previously described (10). The silencing efficiency and specificity of the siRNAs were examined by the supplier. Nontargeting control siRNA was used as control for nonspecific silencing effects.

Statistical analyses. All values presented indicate the mean and standard error of the mean (SEM) unless otherwise indicated. Differences in sMICA levels between treatment groups and control groups were determined using paired, two-tailed Student's *t*-test. *p*-Values less than 0.05 were considered statistically significant.

Results

ATRA inhibited ADAM9 enzymatic activity in vitro. We evaluated ADAM9 activity using a new *in vitro* system presented in Materials and Methods and a library of FDA-approved drugs and identified that ATRA and bexaroten significantly suppressed the enzymatic activity of ADAM9, at 66.7 μM and 57.4 μM, respectively (Figure 1A). Subsequently, the effects were found to be dose-dependent (Figure 1B).

Retinoids suppressed ADAM9 enzymatic activity. Next, the suppressive effects of ATRA analogs and derivatives (*i.e.*,

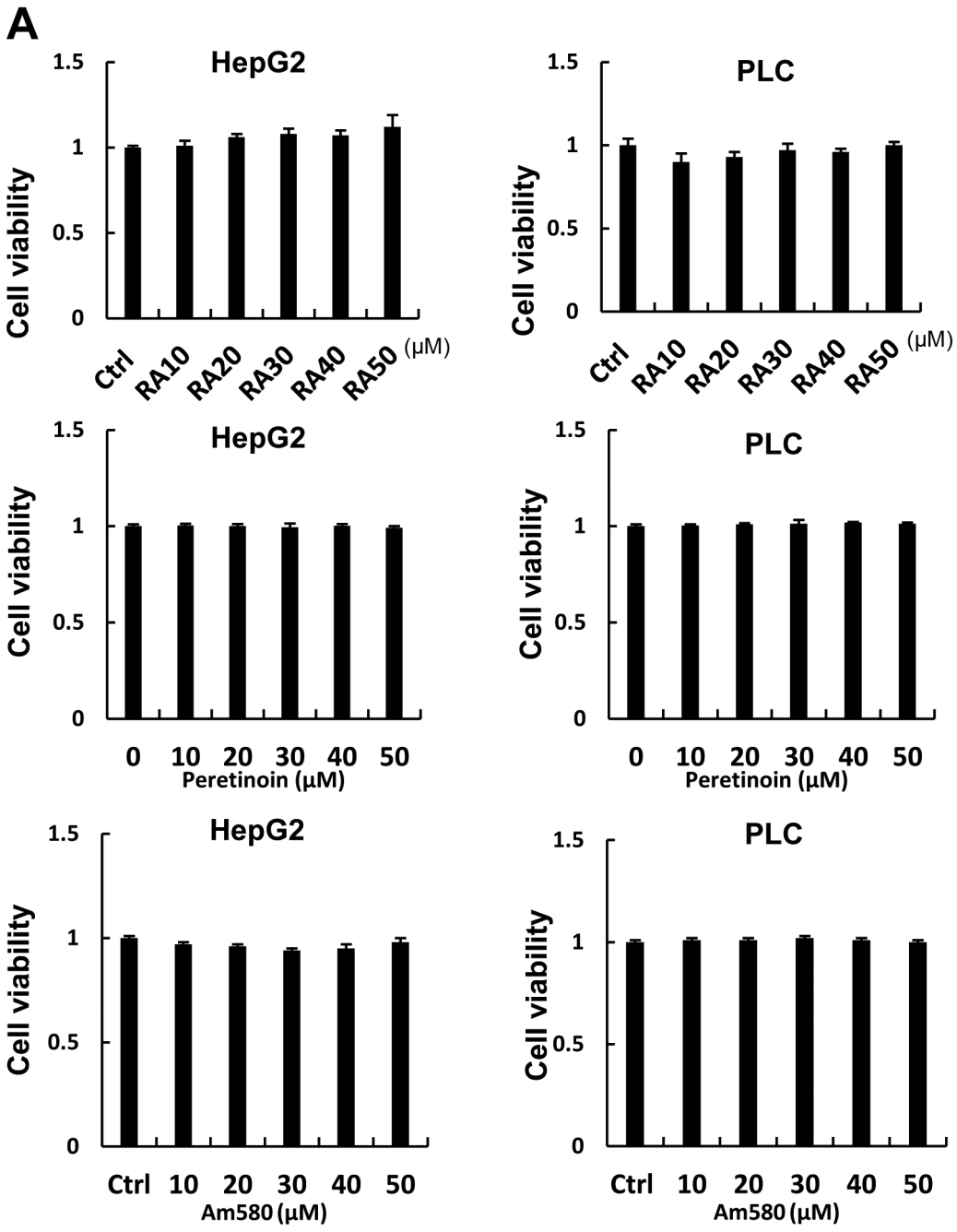


Figure 2. *Continued*

Am580, peretinoin, and 13-cis RA) on the enzymatic activity of ADAM9 were evaluated (Figure 1C).

Interestingly, all showed dose-dependent inhibitory effects on ADAM9 activity (Figure 1D). In contrast, other analogs including acitrecin, 9-cis RA, all-trans retinal, all-trans retinol, retinyl palmitate, and 4-HPR (Figure 1E) did not exert inhibitory effects (data not shown).

Retinoids decreased sMICA levels. To investigate cell biological impact, the effects of retinoids on sMICA in HepG2 and PLC/PRF/5 cells were analyzed. After 48 h of treatment with 10-50 μM ATRA, Am580, peretinoin showed no toxicity (Figure 2A), while these treatments clearly decreased sMICA levels in both HepG2 and PLC/PRF/5 cells (Figure 2B).

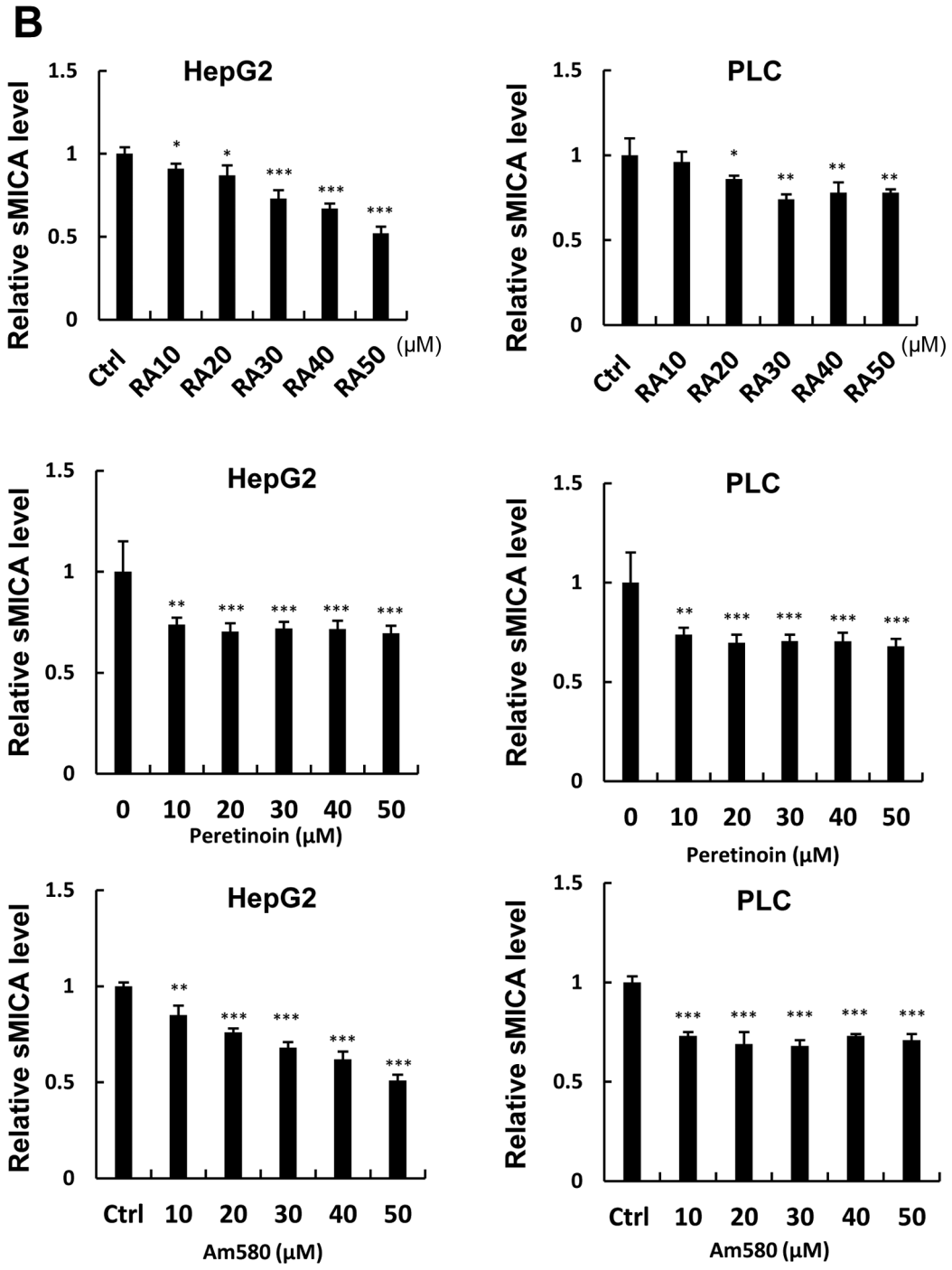


Figure 2. *Continued*

Treatment with the combination of retinoids and regorafenib decreased sMICA levels more than treatment with the single agents. Treatment with 50 μM ATRA or 50 μM Am580 in combination with 2 μM REG significantly decreased sMICA levels as compared to the treatment with the retinoids only (Figure 2C), without significant cytotoxicities in both cell lines (Figure 2D).

Inhibitory effects of retinoids on the enzymatic activity of ADAM10. Subsequently, the effects of 500 μM retinoids on

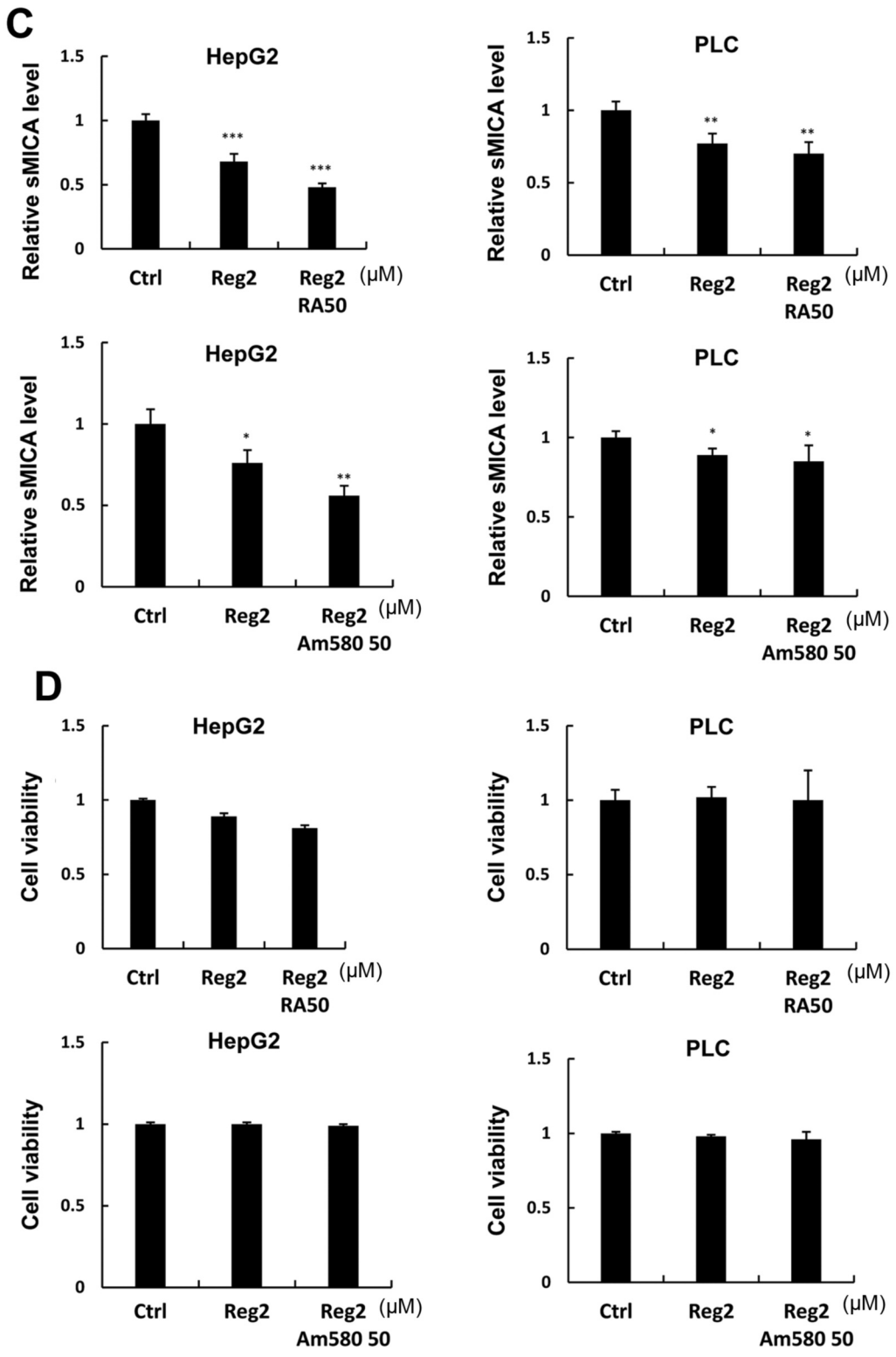


Figure 2. *Combination treatments with retinoids and regorafenib further suppressed soluble MHC class I polypeptide-related sequence A (sMICA) release. HepG2 and PLC/PRF/5 cells were treated with retinoids for 48 h and cell viability (A) and sMICA levels (B) were determined by CCK8 assay and ELISA, respectively. Furthermore, HepG2 and PLC/PRF/5 cells were treated with regorafenib (REG) and ATRA, or Am580 for 48 h, and sMICA levels (C) and cell viabilities (D) were determined by CCK8 assay and ELISA, respectively. *p<0.05; **p<0.01; ***p<0.005. Error bars represent SEM. The results derive from three independent experiments.*

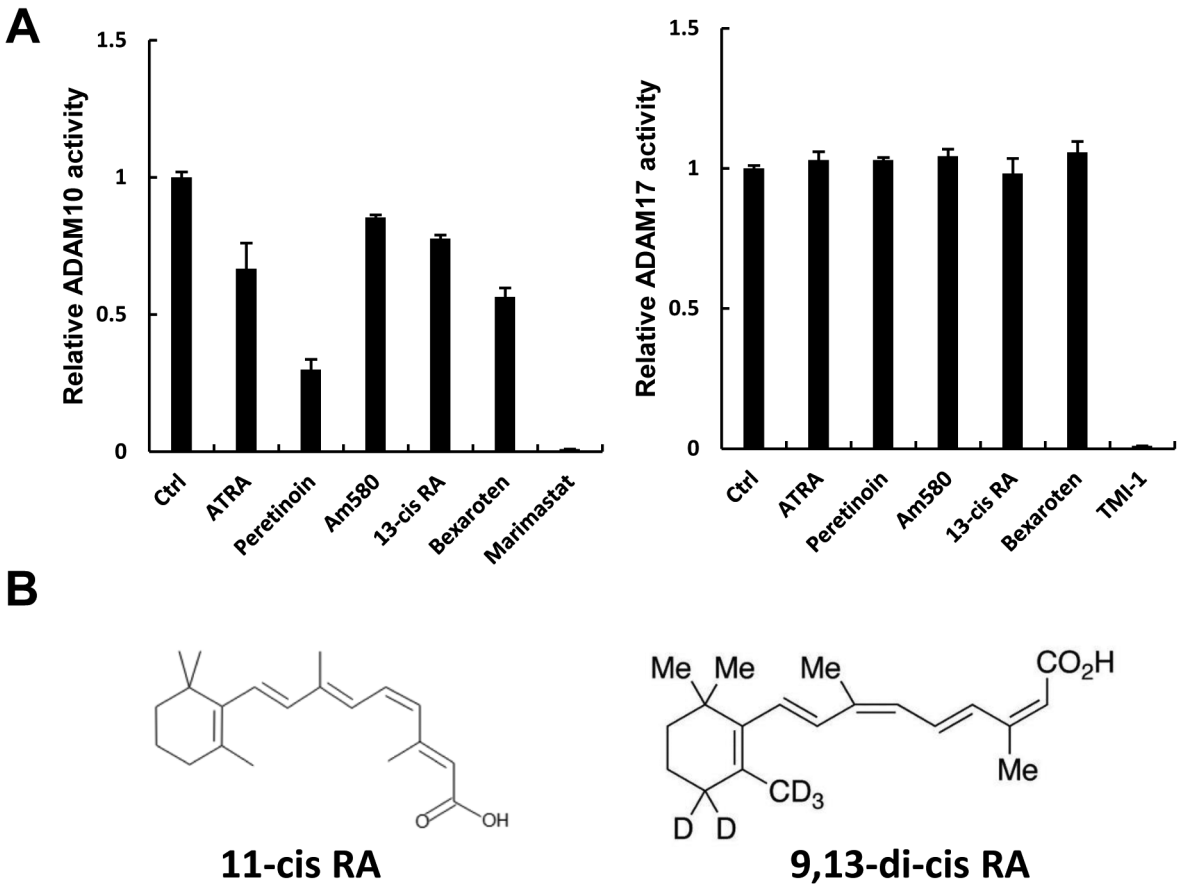


Figure 3. *Continued*

ADAM10 and ADAM17 were also examined *in vitro*. The enzymatic activity of ADAM10 was suppressed, whereas that of ADAM17 was not altered (Figure 3A). Furthermore, treatment with retinoids did not change the mRNA expression of MICA, ADAM9, ADAM10, or ADAM17 (data not shown).

Chemical structural features of retinoic acid responsible for its bioactivity. To dissect the structural features of ATRA responsible for ADAM9 activity suppression, we further evaluated multiple isomers (Figure 3B). 11-cis RA exerted inhibitory effects similarly to 13-cis RA (Figure 1D), and so did 9,13-di-cis-RA (Figure 3C), unlike 9-cis RA (data not shown).

These analogs showed dose-dependent inhibition of ADAM9 and ADAM10 (Figure 3C). Meanwhile, ADAM17 enzymatic activity suppression was not observed at 500 μM. The conformational similarity of 9-cis RA, the least efficacious isomer herein, to ATRA was calculated to be less than the other three RAs' above (data not shown), indicative of the structural analogy to ATRA correlated with inhibitory effects. Thus, the structure-activity relationship (SAR) approach suggested the functional implication of polyenoic chain organization affected by the cis-trans isomerization for enzymatic modulations.

11-cis RA and 9,13-di-cis RA also decreased sMICA levels. Treatment of HepG2 cells with 11-cis RA, and 9,13-di-cis RA for 48 h showed no cytotoxicity, whereas these treatments clearly decreased sMICA levels (Figure 4A).

Retinoids decreased sMICA levels via RXRα. To further clarify the mechanisms of retinoids-mediated suppression of sMICA, the involvement of well-known retinoid receptors related was analyzed. The decrease in sMICA levels by ATRA (Figure 4B) and 9,13-di-cis RA (Figure 4C) was partially and fully negated in the presence of si-retinoid X receptor (RXR)α, respectively, in HepG2 cells, without cytotoxicity. The same effect was not observed with siRXRβ, siRXRγ, si-retinoid A receptor (RAR)α, siRARβ, siRARγ (data not shown).

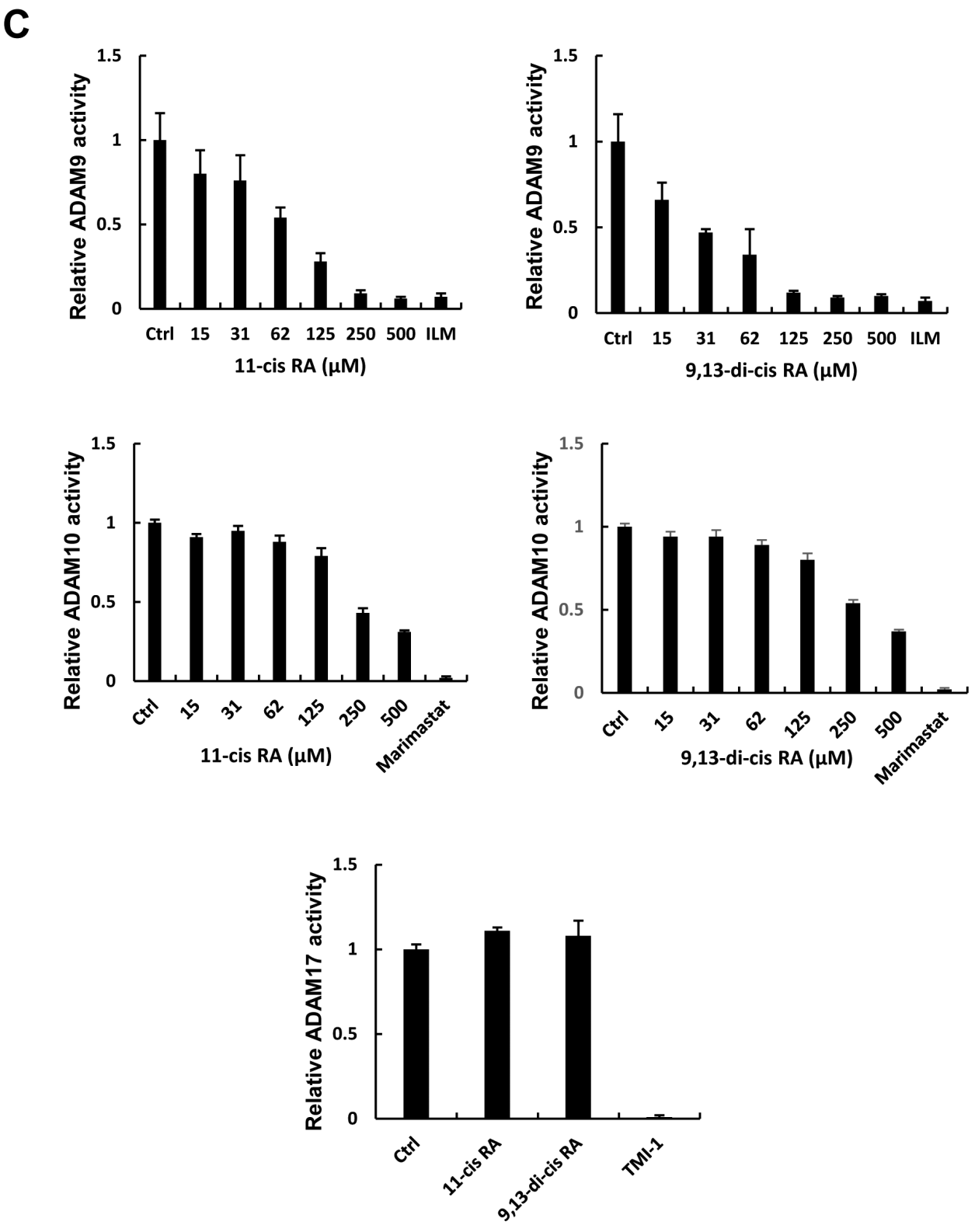
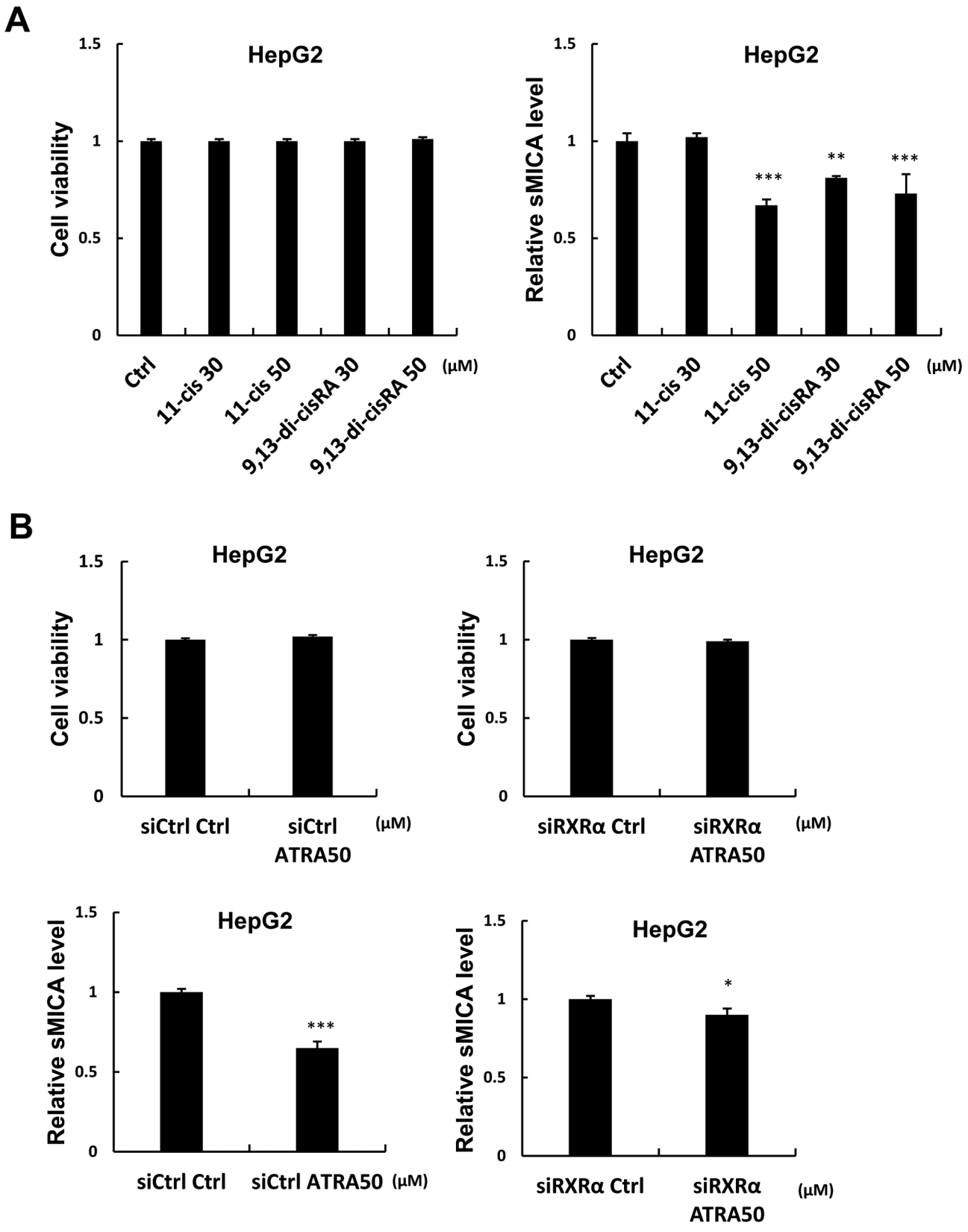


Figure 3. *Impacts of retinoids on enzymatic activity of ADAMs. (A) Enzymatic inhibition of ADAM10 and ADAM17 by 500 μM all-trans retinoic acid (ATRA) analogs in vitro. (B) Molecular structures of ATRA isomers. (C) Enzymatic inhibition of ADAM9, ADAM10, and ADAM17 by 11-cis RA, 9,13-di-cis-RA in vitro. Ilomastat (ILM), marimastat, and TMI-1 are used as the ADAM9, ADAM10, and ADAM17 control inhibitors, respectively. Error bars represent SEM. The results derive from three independent experiments.*



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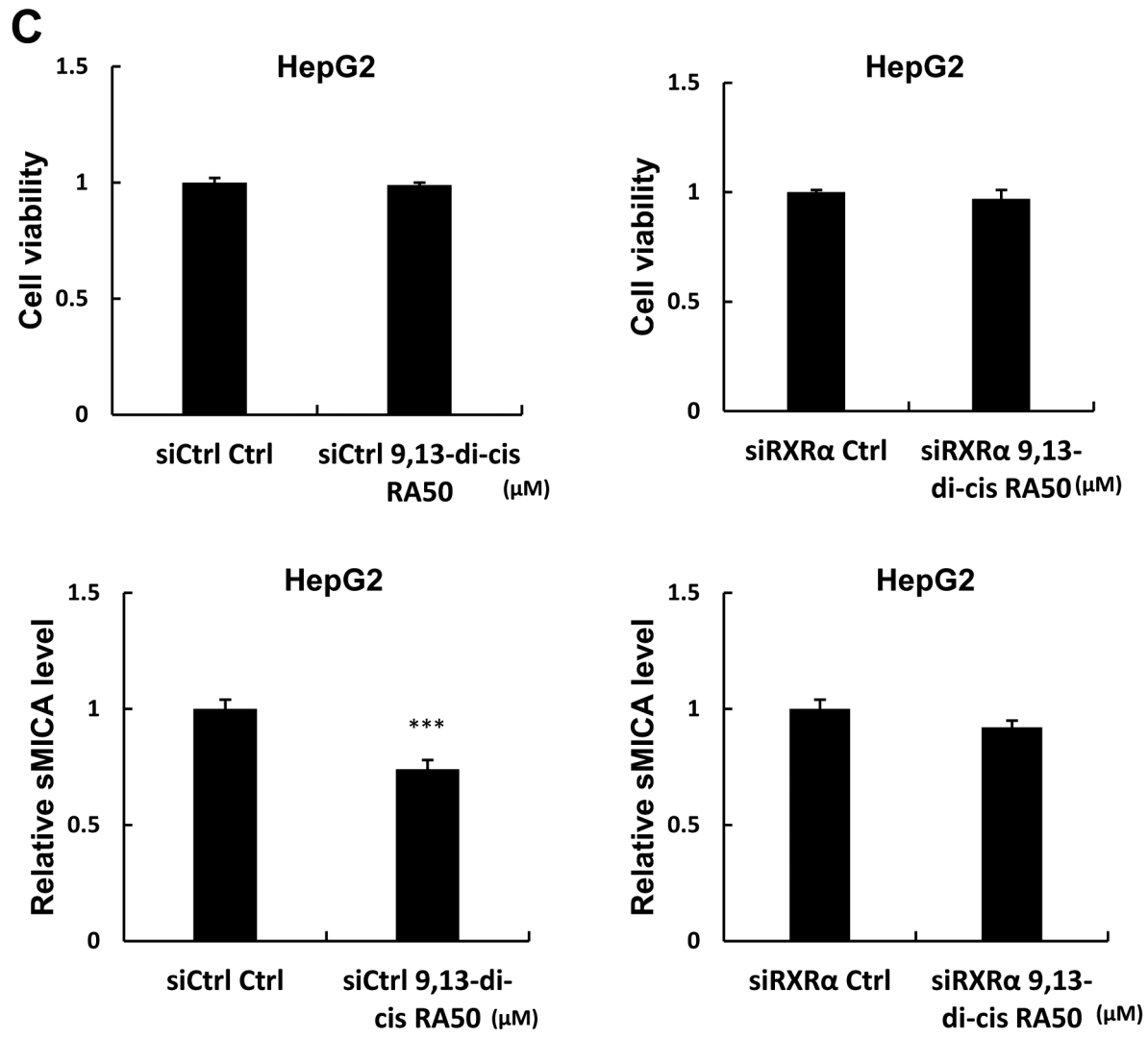

Figure 4. *Retinoids decreased soluble MHC class I polypeptide-related sequence A (sMICA) levels via RXRα. (A) HepG2 cells were treated with 11-cis-RA and 9,13-di-cis RA for 48 h and the cell viabilities and sMICA levels were determined by CCK8 assay and ELISA, respectively. HepG2 cells were transfected with siRNA to RXRαfor 24 h, followed by the treatment with all-trans retinoic acid (ATRA) (B) or 9,13-di-cis RA (C) for 48 h. The cell viabilities and sMICA levels were assessed by CCK8 assay and ELISA, respectively. *p<0.05; **p<0.01; ***p<0.005. Error bars represent SEM. The results derive from three independent experiments.*

Discussion

Among patients with chronic hepatitis C, MICA has been identified as an HCC susceptibility gene in our previous GWAS (9). MICA is a NKG2D ligand, which functions as a "kill-me" signal inducing cytotoxicities to infected or cancerous cells by NK cells (14). mMICA is prone to proteolytic cleavage, and the released sMICA is an immunological decoy in the serum (15). Therefore, the increase in sMICA has been shown to be an indicator of poor prognosis in patients with chronic hepatitis or hepatitis B (16). In other cohorts with chronic hepatitis C patients, higher sMICA levels after viral eradication were associated with HCC development (17).

Suppression of ADAM9 activity has been shown to enhance the NK cell cytotoxicity to HCC by MICA upregulation (13, 18). ADAM9 has also been reported to be over-expressed in the cancer microenvironment cells, including blood vessels and immune cells, of several cancer types including liver cancer (19). Interactions between these components of the microenvironment enhance tumor metastasis and epithelial-mesenchymal transition (20).

Jinushi M *et al*. have previously shown that ATRA affects MICA expression; however, sMICA concentration was not

evaluated (21). In this study, we screened *in vitro* a library of FDA-approved drugs to identify potent inhibitors of ADAM9 and discovered that retinoids (*i.e.*, ATRA, peretinoin, and Am580) suppressed ADAM9 activity (Figure 1A and 1B).

Among known MICA sheddases in HCC (10), enzymatic suppression of ADAM10 but not of ADAM17 was observed *in vitro* (Figure 3C).

On the other hand, inhibiting ADAM9 by decreasing its transcription (13, 18) could also be a useful approach for cancer treatment as shown by the multi-kinase inhibitor REG. In reality, the treatment with the combination of REG and retinoids significantly decreased sMICA concentration more than treatment with each retinoid alone (Figure 2C).

At present, peretinoin is under clinical trials to suppress the recurrence of HCC after hepatic resection or radiofrequency ablation and induction of apoptosis is reportedly the action mechanism (6, 22). Our present data also implies that peretinoin could facilitate innate immunity to effectively remove HCC cells and reduce sMICA levels. Further analyses to evaluate its effectiveness based on sMICA concentrations in patients with chronic hepatitis would clarify its potential as a biomarker and predict its effect in advance.

Notably, ADAM9 is a putative therapeutic target in HCC because of its role in the immune microenvironment and cancer development. Furthermore, previous studies have demonstrated the relationship between ADAM9 expression and clinicopathological features including disease prognosis, short overall survival, tumor grade, metastasis, and the development of resistance in various cancers including HCC (19). Potentially, ADAM9 expression could affect the overall survival of patients with HCC *via* MICA shedding (13, 18).

Okita K *et al*. have reported the results of a randomized double-blind placebo-controlled study with peretinoin after curative therapy of hepatitis C-related HCC. In that trial, administration of 600 mg/day peretinoin was shown to be the optimal dose, and treatment might reduce the 2-year recurrence of hepatitis C-related HCC (23). While oral administration of peretinoin did not prevent local recurrence, a second primary hepatoma was prevented after surgical resection of the original tumor or the percutaneous injection of ethanol with four years of survey (4).

Further sub-group analysis comparing overall survival among patients classified as Child-Pugh A revealed that survival of the 600 mg/day group was significantly longer than that of the placebo group (24). Hence, a new clinical phase III trial is currently underway to examine the effects of peretinoin on controlling HCC recurrence among those classified as Child-Pugh A who have completed curative treatment for HCV-HCC. The expression of ADAM9 and ADAM10 in HCC or sMICA concentration in the sera of patients are potential biomarkers for predicting the treatment efficacy with peretinoin. Suppression of ADAM10 enzymatic activity has also been shown to enhance NK cell cytotoxicity against HCC by decreasing MICA shedding (25).

Based on the previous experiments, RXRα is thought to be one of the most important retinoid receptors regarding the regulation of fundamental cell activities, including normal cell proliferation and metabolism, and acts as the master regulator of nuclear receptors (26). In hepatitis C viral infection, the core protein binds with RXRα, resulting in the upregulation of some lipid metabolism enzymes and potentially contributes to liver injury and hepatocarcinogenesis (27). Furthermore, in HCC, Shimizu *et al*. have reported that a malfunction of RXRα due to phosphorylation by the Ras mitogen activated protein kinase signaling pathway is profoundly associated with the development of HCC and thus may be a critical target for HCC chemoprevention (28). Our study also shows the benefit that derives from RXRα-mediated suppression of the shedding of MICA, and subsequent activation of innate immunity against hepatocarcinogenesis.

This is the first report to describe the molecular mechanisms of modulation of MICA expression by retinoids. Retinoids suppress the enzymatic function of ADAM9 and ADAM10, subsequently inhibiting MICA shedding. However, a detailed clinical investigation in patients with advanced HCC is required. The improved potency of peretinoin in patients with HCC could be partially attributed to suppression of ADAM9 function and HCC progression. Peretinoin may also be an important agent to decrease sMICA levels for the reduction of immunological decoy and restoration of innate immunity against HCC, which is expected to be analyzed in ongoing clinical trials. SAR analyses on ATRA and isomers indicated the significance of polyene chain structures with the polar end group stretched and coordinated conformationally for enzymatic inhibition *in vitro*. This observation was supported by attenuation of the inhibitory effects by chain modifications including cyclization, as in the case of bexaroten and AM580 (Figure 1C and 3B). Indeed, further detailed investigations into individual retinoids' modes of molecular interactions (29) with ADAMs are warranted. Chemical structure and biochemical insights would also directly contribute to our understanding of the development and utilization of retinoids for interventional purposes. In addition, the multi-kinase inhibitor REG has been reported to improve outcomes in HCC patients with sorafenib (SOR)-resistant disease in the RESORCE trial (30). Our previous study has corroborated that REG potentiates immune-mediated HCC cell death by decreasing sMICA concentration to a greater extent than SOR, by mainly targeting ADAM9 transcriptionally (13). Overall, our results may be useful for developing strategies for novel HCC treatments. Furthermore, retinoids may also be used for immunological control of HCC because they suppress ADAM9 enzymatic activity and increase treatment efficacy in combination with conventional multi-kinase inhibitors.

Conflicts of Interest

The Authors declare that they have no conflicts of interest in relation to this study.

Authors' Contributions

Y.O and J.A: research design and discussion, drug screen and experimentation, data analysis, and manuscript writing; K.G: research design, discussion, and data analysis; H.N: research discussion and experimentation, and data analysis; R.N, R.M, I.S, Y.N, A.K, M.T, Y.I, S.U, Y.S, M.U, M.S, and H.Y: research discussion; N.K: research design and discussion; All Authors read and approved the final manuscript.

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References

1 Fujiwara N, Friedman SL, Goossens N and Hoshida Y: Risk factors and prevention of hepatocellular carcinoma in the era of precision medicine. J Hepatol *68(3)*: 526-549, 2018. PMID: 28989095. DOI: 10.1016/j.jhep.2017.09.016

2 Kaneko S, Ikeda K, Matsuzaki Y, Furuse J, Minami H, Okayama Y, Sunaya T, Ito Y, Inuyama L and Okita K: Safety and effectiveness of sorafenib in Japanese patients with hepatocellular carcinoma in daily medical practice: Interim analysis of a prospective postmarketing all-patient surveillance study. J Gastroenterol *51(10)*: 1011-1021, 2016. PMID: 26931117. DOI: 10.1007/s00535-016-1173-5

3 Nakagawa S, Wei L, Song WM, Higashi T, Ghoshal S, Kim RS, Bian CB, Yamada S, Sun X, Venkatesh A, Goossens N, Bain G, Lauwers GY, Koh AP, El-Abtah M, Ahmad NB, Hoshida H, Erstad DJ, Gunasekaran G, Lee Y, Yu ML, Chuang WL, Dai CY, Kobayashi M, Kumada H, Beppu T, Baba H, Mahajan M, Nair VD, Lanuti M, Villanueva A, Sangiovanni A, Iavarone M, Colombo M, Llovet JM, Subramanian A, Tager AM, Friedman SL, Baumert TF, Schwarz ME, Chung RT, Tanabe KK, Zhang B, Fuchs BC, Hoshida Y and Precision Liver Cancer Prevention Consortium: Molecular liver cancer prevention in cirrhosis by organ transcriptome analysis and lysophosphatidic acid pathway inhibition. Cancer Cell *30(6)*: 879-890, 2016. PMID: 27960085. DOI: 10.1016/j.ccell.2016.11.004

4 Muto Y, Moriwaki H, Ninomiya M, Adachi S, Saito A, Takasaki KT, Tanaka T, Tsurumi K, Okuno M, Tomita E, Nakamura T and 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 *334(24)*: 1561-1567, 1996. PMID: 8628336. DOI: 10.1056/NEJM199606133342402

5 Boone CW, Kelloff GJ and Malone WE: Identification of candidate cancer chemopreventive agents and their evaluation in animal models and human clinical trials: A review. Cancer Res *50(1)*: 2-9, 1990. PMID: 2403415.

6 Okada H, Honda M, Campbell JS, Sakai Y, Yamashita T, Takebuchi Y, Hada K, Shirasaki T, Takabatake R, Nakamura M, Sunagozaka H, Tanaka T, Fausto N and Kaneko S: Acyclic retinoid targets platelet-derived growth factor signaling in the prevention of hepatic fibrosis and hepatocellular carcinoma development. Cancer Res *72(17)*: 4459-4471, 2012. PMID: 22651928. DOI: 10.1158/0008-5472.CAN-12-0028

7 Guan HB, Nie YZ, Zheng YW, Takiguchi K, Yu HW, Zhang RR, Li B, Tsuchida T and Taniguchi H: Acyclic retinoid induces differentiation and apoptosis of murine hepatic stem cells. Stem Cell Res Ther *6*: 51, 2015. PMID: 25881300. DOI: 10.1186/s13287-015-0046-9

8 Guerra N, Tan YX, Joncker NT, Choy A, Gallardo F, Xiong N, Knoblaugh S, Cado D, Greenberg NM and Raulet DH: NKG2D-deficient mice are defective in tumor surveillance in models of spontaneous malignancy. Immunity *28(4)*: 571-580, 2008. PMID: 18394936. DOI: 10.1016/j.immuni.2008.02.016

9 Kumar V, Kato N, Urabe Y, Takahashi A, Muroyama R, Hosono N, Otsuka M, Tateishi R, Omata M, Nakagawa H, Koike K, Kamatani N, Kubo M, Nakamura Y and Matsuda K: Genome-wide association study identifies a susceptibility locus for HCV-induced hepatocellular carcinoma. Nat Genet *43(5)*: 455-458, 2011. PMID: 21499248. DOI: 10.1038/ng.809

10 Arai J, Goto K, Tanoue Y, Ito S, Muroyama R, Matsubara Y, Nakagawa R, Kaise Y, Lim LA, Yoshida H and Kato N: Enzymatic inhibition of MICA sheddase ADAM17 by lomofungin in hepatocellular carcinoma cells. Int J Cancer *143(10)*: 2575-2583, 2018. PMID: 29873070. DOI: 10.1002/ijc.31615

11 Hu D, Shen D, Zhang M, Jiang N, Sun F, Yuan S and Wan K: MiR-488 suppresses cell proliferation and invasion by targeting ADAM9 and lncRNA HULC in hepatocellular carcinoma. Am J Cancer Res *7(10)*: 2070-2080, 2017. PMID: 29119055.

12 Arai J, Goto K, Otoyama Y, Nakajima Y, Sugiura I, Kajiwara A, Tojo M, Ichikawa Y, Uozumi S, Shimozuma Y, Uchikoshi M, Sakaki M, Nozawa H, Nakagawa R, Muroyama R, Kato N and Yoshida H: Leukotriene receptor antagonists enhance HCC treatment efficacy by inhibiting ADAMs and suppressing MICA shedding. Cancer Immunol Immunother *70(1)*: 203-213, 2021. PMID: 32683508. DOI: 10.1007/s00262-020-02660-2

13 Arai J, Goto K, Stephanou A, Tanoue Y, Ito S, Muroyama R, Matsubara Y, Nakagawa R, Morimoto S, Kaise Y, Lim LA, Yoshida H and Kato N: Predominance of regorafenib over sorafenib: Restoration of membrane-bound MICA in hepatocellular carcinoma cells. J Gastroenterol Hepatol *33(5)*: 1075-1081, 2018. PMID: 29055152. DOI: 10.1111/jgh.14029

14 Goto K and Kato N: MICA SNPs and the NKG2D system in virus-induced HCC. J Gastroenterol *50(3)*: 261-272, 2015. PMID: 25270965. DOI: 10.1007/s00535-014-1000-9

15 Kohga K, Takehara T, Tatsumi T, Miyagi T, Ishida H, Ohkawa K, Kanto T, Hiramatsu N and Hayashi N: Anticancer chemotherapy inhibits MHC class I-related chain a ectodomain shedding by downregulating ADAM10 expression in hepatocellular carcinoma. Cancer Res *69(20)*: 8050-8057, 2009. PMID: 19826051. DOI: 10.1158/0008-5472.CAN-09-0789

16 Kumar V, Yi Lo PH, Sawai H, Kato N, Takahashi A, Deng Z, Urabe Y, Mbarek H, Tokunaga K, Tanaka Y, Sugiyama M, Mizokami M, Muroyama R, Tateishi R, Omata M, Koike K, Tanikawa C, Kamatani N, Kubo M, Nakamura Y and Matsuda K: Soluble MICA and a MICA variation as possible prognostic biomarkers for HBV-induced hepatocellular carcinoma. PLoS One *7(9)*: e44743, 2012. PMID: 23024757. DOI: 10.1371/journal.pone.0044743

17 Huang CF, Wang SC, Yeh ML, Huang CI, Tsai PC, Lin ZY, Chen SC, Dai CY, Huang JF, Chuang WL, Chen A and Yu ML: Association of serial serum major histocompatibility complex class I chain-related A measurements with hepatocellular carcinoma in chronic hepatitis C patients after viral eradication. J Gastroenterol Hepatol *34(1)*: 249-255, 2019. PMID: 29932235. DOI: 10.1111/jgh.14359

18 Kohga K, Takehara T, Tatsumi T, Ishida H, Miyagi T, Hosui A and Hayashi N: Sorafenib inhibits the shedding of major histocompatibility complex class I-related chain A on hepatocellular carcinoma cells by down-regulating a disintegrin and metalloproteinase 9. Hepatology *51(4)*: 1264-1273, 2010. PMID: 20099300. DOI: 10.1002/hep.23456

19 Oria VO, Lopatta P and Schilling O: The pleiotropic roles of ADAM9 in the biology of solid tumors. Cell Mol Life Sci *75(13)*: 2291-2301, 2018. PMID: 29550974. DOI: 10.1007/s00018-018-2796-x

20 Chou CW, Huang YK, Kuo TT, Liu JP and Sher YP: An overview of ADAM9: Structure, activation, and regulation in human diseases. Int J Mol Sci *21(20)*: 7790, 2020. PMID: 33096780. DOI: 10.3390/ijms21207790

21 Jinushi M, Takehara T, Tatsumi T, Kanto T, Groh V, Spies T, Kimura R, Miyagi T, Mochizuki K, Sasaki Y and Hayashi N: Expression and role of MICA and MICB in human hepatocellular carcinomas and their regulation by retinoic acid. Int J Cancer *104(3)*: 354-361, 2003. PMID: 12569559. DOI: 10.1002/ijc.10966

22 Tatsukawa H, Sano T, Fukaya Y, Ishibashi N, Watanabe M, Okuno M, Moriwaki H and Kojima S: Dual induction of caspase 3- and transglutaminase-dependent apoptosis by acyclic retinoid in hepatocellular carcinoma cells. Mol Cancer *10*:4, 2011. PMID: 21214951. DOI: 10.1186/1476-4598-10-4

23 Okita K, Izumi N, Matsui O, Tanaka K, Kaneko S, Moriwaki H, Ikeda K, Osaki Y, Numata K, Nakachi K, Kokudo N, Imanaka K, Nishiguchi S, Okusaka T, Nishigaki Y, Shiomi S, Kudo M, Ido K, Karino Y, Hayashi N, Ohashi Y, Makuuchi M, Kumada H and Peretinoin Study Group: Peretinoin after curative therapy of hepatitis C-related hepatocellular carcinoma: A randomized double-blind placebo-controlled study. J Gastroenterol *50(2)*: 191-202, 2015. PMID: 24728665. DOI: 10.1007/s00535-014-0956-9

24 Okita K, Izumi N, Ikeda K, Osaki Y, Numata K, Ikeda M, Kokudo N, Imanaka K, Nishiguchi S, Kondo S, Nishigaki Y, Shiomi S, Ueshima K, Isoda N, Karino Y, Kudo M, Tanaka K, Kaneko S, Moriwaki H, Makuuchi M, Okusaka T, Hayashi N, Ohashi Y, Kumada H and Peretinoin Study Group: Survey of survival among patients with hepatitis C virus-related hepatocellular carcinoma treated with peretinoin, an acyclic retinoid, after the completion of a randomized, placebo-controlled trial. J Gastroenterol *50(6)*: 667-674, 2015. PMID: 25209978. DOI: 10.1007/s00535-014-0996-1

25 Goto K, Arai J, Stephanou A and Kato N: Novel therapeutic features of disulfiram against hepatocellular carcinoma cells with inhibitory effects on a disintegrin and metalloproteinase 10. Oncotarget *9(27)*: 18821-18831, 2018. PMID: 29721164. DOI: 10.18632/oncotarget.24568

26 Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schütz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P and Evans RM: The nuclear receptor superfamily: The second decade. Cell *83(6)*: 835-839, 1995. PMID: 8521507. DOI: 10.1016/0092-8674(95)90199-x

27 Koike K: Steatosis, liver injury, and hepatocarcinogenesis in hepatitis C viral infection. J Gastroenterol *44(Suppl 19)*: 82-88, 2009. PMID: 19148799. DOI: 10.1007/s00535-008-2276-4

28 Shimizu M, Takai K and 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 *100(3)*: 369-374, 2009. PMID: 19068086. DOI: 10.1111/j.1349-7006.2008.01045.x

29 Leblanc BP and Stunnenberg HG: 9-cis retinoic acid signaling: Changing partners causes some excitement. Genes Dev *9(15)*: 1811-1816, 1995. PMID: 7649469. DOI: 10.1101/gad.9.15.1811

30 Bruix J, Qin S, Merle P, Granito A, Huang YH, Bodoky G, Pracht M, Yokosuka O, Rosmorduc O, Breder V, Gerolami R, Masi G, Ross PJ, Song T, Bronowicki JP, Ollivier-Hourmand I, Kudo M, Cheng AL, Llovet JM, Finn RS, LeBerre MA, Baumhauer A, Meinhardt G, Han G and RESORCE Investigators: Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): A randomised, double-blind, placebo-controlled, phase 3 trial. Lancet *389(10064)*: 56-66, 2017. PMID: 27932229. DOI: 10.1016/S0140-6736(16)32453-9

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