3. Pour, P.; Kruger, F.W.; Althoff, J.; Cardesa, A.; Mohr, U. Cancer of the pancreas induced in the Syrian golden hamster. *Am. J. Pathol.* **1974**, *76*, 349-358.
4. Pour, P.; Althoff, J.; Krüger, F.W.; Mohr, U. A potent pancreatic carcinogen in Syrian hamsters: *N*-nitrosobis(2-oxopropyl)amine. *J. Natl. Cancer Inst.* **1977**, *58*, 1449-1453.
5. Pour, P. Experimental pancreatic ductal (ductular) tumors. *Monogr. Pathol.* **1980**, *21*, 111-139.
6. Pour, P.M.; Runge, R.G.; Birt, D.; Gingell, R.; Lawson, T.; Nagel, D.; Wallcave, L.; Salmasi, A.H. Current knowledge of pancreatic carcinogenesis in the hamster and its relevance to the human disease. *Cancer* **1981**, *47*, 1573-1587.
7. Pour, P.; Althoff, J.; Takahashi, M. Early lesions of pancreatic ductal carcinoma in the hamster model. *Am. J. Pathol.* **1977**, *88*, 291-308.
8. Schüller, H.M.; Jorquera, R.; Reichert, A.; Castonguay, A. Transplacental induction of pancreas tumors in hamsters by ethanol and the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Cancer Res.* **1993**, *53*, 2498-2501.
9. Longnecker, D.S.; Curphey, T.J. Adenocarcinoma of the pancreas in azaserine-treated rats. *Cancer Res.* **1975**, *35*, 2249-2258.
10. Longnecker, D.S.; Curphey, T.J.; Lilja, H.S.; French, J.I.; Daniel, D.S. Carcinogenicity in rats of the nitrosourea amino acid *N* delta-(*N*-methyl-*N*-nitrosocarbamoyl)-L-ornithine. *Environ. Pathol. Toxicol.* **1980**, *4*, 117-129.
11. Longnecker, D.S.; Curphey, T.J.; Kuhlmann, E.T.; Schaeffer, B.K. Experimental induction of pancreatic carcinomas in the hamster with *N* delta-(*N*-methyl-*N*-nitrosocarbamoyl)-L-ornithine. *J. Natl. Cancer Inst.* **1983**, *71*, 1327-1336.
12. Pour, P.; Salmasi, S.; Runge, R.; Gingell, R.; Wallcave, L.; Nagel, D.; Stepan, K. Carcinogenicity of *N*-nitrosobis(2-hydroxypropyl)amine and *N*-nitrosobis(2-oxopropyl)amine in MRC rats. *J. Natl. Cancer Inst.* **1979**, *63*, 181-190.
13. Sakano, K.; Takahashi, M.; Mutoh, M.; Niho, N.; Komiya, M.; Sato, H.; Tanaka, T.; Sugimura, T.; Wakabayashi, K. Enhanced thyroid carcinogenicity of *N*-nitrosobis(2-oxopropyl)amine in Otsuka Long-Evans Tokushima Fatty rats, a model of type II diabetes mellitus. *Carcinogenesis* **2007**, *28*, 2193-2198.
14. Fujii, K.; Hayakawa, T.; Kikuchi, M. Tumor induction in mice administered neonatally with bis(2-oxopropyl)nitrosamine. *Tohoku J. Exp. Med.* **1994**, *174*, 361-368.
15. Scarpelli, D.G.; Rao, M.S.; Reddy, J.K. Studies of pancreatic carcinogenesis in different animal models. *Environ. Health Persp.* **1984**, *56*, 219-227.
16. Rao, M.S. Animal models of exocrine pancreatic carcinogenesis. *Cancer Metast. Rev.* **1987**, *6*, 665-676.
17. Longnecker, D. Experimental pancreatic cancer: Role of species, sex and diet. *Bull. Cancer* **1990**, *77*, 27-37.
18. Dissin, J.; Mills, L.R.; Mains, D.L.; Black, O., Jr.; Webster, P.D., 3rd. Experimental induction of pancreatic adenocarcinoma in rats. *J. Natl. Cancer Inst.* **1975**, *55*, 857-864.
19. Bockman, D.E.; Black, O., Jr.; Mills, L.R.; Mainz, D.L.; Webster, P.D. 3rd. Fine structure of pancreatic adenocarcinoma induced in rats by 7,12-dimethylbenz(a)anthracene. *J. Natl. Cancer Inst.* **1976**, *57*, 931-936.

20. Osvaldt, A.B.; Wendt, L.R.; Bersch, V.P.; Backes, A.N.; de Cássia, A.; Schumacher, R.; Edelweiss, M.I.; Rohde, L. Pancreatic intraepithelial neoplasia and ductal adenocarcinoma induced by DMBA in mice. *Surgery* **2006**, *140*, 803-809.
21. Jimenez, R.E.; Z'graggen, K.; Hartwig, W.; Graeme-Cook, F.; Warshaw, A.L.; Castillo, C.F. Immunohistochemical characterization of pancreatic tumors induced by dimethylbenzanthracene in rats. *Am. J. Pathol.* **1999**, *154*, 1223-1229.
22. Z'graggen K.; Warshaw, A.L.; Welner, J.; Graeme-Cook, F.; Jimenez, R.E.; Castillo, C.F. Promoting effects of a high-fat/high-protein diet in DMBA-induced ductal pancreatic cancer in rats. *Ann. Surgery* **2001**, *233*, 688-695.
23. Hruban, R.H.; Adsay, N.V.; Albores-Saavedra, J.; Anver, M.R.; Biankin, A.V.; Boivin, G.P.; Furth, E.E.; Furukawa, T.; Klein, A.; Klimstra, D.S.; Kloppel, G.; Lauwers, G.Y.; Longnecker, D.S.; Luttges, J.; Maitra, A.; Offerhaus, G.J.; Pérez-Gallego, L.; Redston, M.; Tuveson, D.A. Pathology of genetically engineered mouse models of pancreatic exocrine cancer: Consensus report and recommendations. *Cancer Res.* **2006**, *66*, 95-106.
24. Grippo, P.J.; Nowlin, P.S.; Demeure, M.J.; Longnecker, D.S.; Sandgren, E.P. Preinvasive pancreatic neooplasia of ductal phenotype induced by acinar cell targeting of mutant Kras in transgenenic mice. *Cancer Res.* **2003**, *63*, 2016-2019.
25. Hingorani, S.R.; Petricoin, E.F.; Maitra, A.; Rajapakse, V.; King, C.; Jacobetz, M.A.; Ross, S.; Conrads, T.P.; Veenstra, T.D.; Hitt, B.A.; Kawaguchi, Y.; Johann, D.; Liotta, L.A.; Crawford, H.C.; Putt, M.E.; Jacks, T.; Wright, C.V.; Hruban, R.H.; Lowy, A.M.; Tuveson, D.A. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell* **2003**, *4*, 437-450.
26. Deramaudt, T.; Rustgi, A.K. Mutant KRAS in the initiation of pancreatic cancer. *Biochem. Biophys. Acta* **2005**, *1756*, 97-101.
27. Aguirre, A.J.; Bardeesy, N.; Sinha, M.; Lopez, L.; Tuveson, D.A.; Horner, J.; Redston, M.S., DePinho, R.A. Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adeocarcinoma. *Genes & Dev.* **2003**, *17*, 3112-3126.
28. Hingorani, S.R.; Wang, L.; Multani, A.S.; Combs, C.; Deramaudt, T.B.; Hruban, R.H.; Rustgi, A.K.; Chang, S.; Tuveson, D.A. *Trp53*^{R172H} and *Kras*^{G12D} cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. *Cancer Cell* **2005**, *7*, 469-483.
29. Kijima, K.; Vickers, S.M.; Adsay, N.V.; Jhala, N.C.; Kim, H.; Schoeb, T.R.; Grizzle, W.E.; Klug, C.A. Inactivation of Smad4 accelerates KrasG12D-mediated pancreatic neoplasia. *Cancer Res.* **2007**, *67*, 8121-8130.
30. Ijichi, H.; Chytil, A.; Gorska, A.E.; Aakre, M.E.; Fujitani, Y.; Fujitani, S.; Wright, C.V.E.; Moses, H.L. Aggressive pancreatic ductal adenocarcinoma in mice caused by pancreas-specific blockade of transforming growth factor-beta signaling in cooperation with active Kras exoression. *Genes Dev.* **2006**, *20*, 3147-3160.
31. Guerra, C.; Schuhmacher, A.J.; Cañamero, M.; Grippo, P.J.; Verdaguer, L.; Pérez-Gallego, L.; Dubus, P.; Sandgren, E.P.; Barbacid, M. Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-*Ras* oncogenes in adult mice. *Cancer Cell* **2007**, *11*, 291-302.

32. Ueda, S.; Fukamachi, K.; Matsuoka, Y.; Takasuka, N.; Takeshita, F.; Naito, A.; Iigo, M.; Alexander D.B.; Moore, M.A.; Saito, I.; Ochiya, T.; Tsuda, H. Ductal origin of pancreatic adenocarcinomas induced by conditional activation of a human Ha-ras oncogene in rat pancreas. *Carcinogenesis* **2006**, *27*, 2497-2510.
33. Fukamachi, K.; Tanaka, H.; Hagiwara, Y.; Ohara, H.; Joh, T.; Iigo, M.; Alexander, D.B.; Xu, J.; Long, N.; Takigahira, M.; Yanagihara, Y.; Hino, O.; Saito, I.; Tsuda, H. An animal model of preclinical diagnosis of pancreatic ductal carcinomas. *Biochem. Biophys. Res. Commun.* **2009**, *390*, 636-641.
34. Hruban, R.H.; Wilentz, R.E.; Kern, S.E. Genetic progression in the pancreatic ducts. *Am. J. Pathol.* **2000**, *156*, 1821-1825.
35. Bardeesy, N.; DePinho, R.A. Pancreatic cancer biology and genetics. *Nature Rev.* **2002**, *2*, 897-909.
36. Moore, P.; Beghelli, S.; Zamboni, G.; Scarpa, A. Genetic abnormalities in pancreatic cancer. *Mol. Cancer* **2003**, *2*, 1-7.
37. Tarafa, G.; Villanueva, A.; Farré, L.; Rodríguez, J., Musulén, E.; Reyes, G.; Seminago, R.; Olmedo, E.; Paules, A.B.; Peinado, M.A.; Bachs, O.; Capellá, G. DCC and SMAD4 alterations in human colorectal and pancreatic tumor dissemination. *Oncogene* **2000**, *19*, 546-555.
38. Konishi, Y.; Tsutsumi, M.; Tsujiuchi, T. Mechanistic analysis of pancreatic ductal carcinogenesis in hamsters. *Pancreas* **1998**, *16*, 300-306.
39. Tsujiuchi, T.; Tsutsumi, M.; Konishi, Y. Molecular aspects during multi-step chemical induced carcinogenesis in the lung and pancreas. *J. Toxicol. Pathol.* **2003**, *16*, 133-138.
40. van Kranen, H.J.; Vermeulen, E.; Schoren, L.; Bax, J.; Woutersen, R.A.; van Iersel, P.; van Kreijl, C.F.; Scherer, E. Activation of c-K-*ras* is frequent in pancreatic carcinomas of Syrian hamsters, but is absent in pancreatic tumors of rats. *Carcinogenesis* **1991**, *12*, 1477-1482.
41. Cerny, W.L.; Mangold, K.A.; Scarpelli, D.G. K-*ras* mutation is an early event in pancreatic duct carcinogenesis in the Syrian golden hamster. *Cancer Res.* **1992**, *52*, 4507-4513.
42. Chang, K.W.; Laconi, S.; Mangold, K.A.; Hubchak, S.; Scarpelli, D.G. Multiple genetic alterations in hamster pancreatic ductal adenocarcinomas. *Cancer Res.* **1995**, *55*, 2560-2568.
43. Burmer, G.C.; Rabinovitch, P.S.; Loeb, L.A. Frequency and spectrum of c-Ki-ras mutations in human sporadic colon carcinoma, carcinomas arising in ulcerative colitis, and pancreatic adenocarcinoma. *Environ. Health Persp.* **1991**, *93*, 27-31.
44. Mu, D.; Peng, Y.; Xu, Q. Values of mutations of K-*ras* oncogene at codon 12 in detection of pancreatic cancer: 15-year experience. *World J. Gastroenterol.* **2004**, *10*, 471-475.
45. Saif, M.W.; Karapanagiotou, L.; Syrigos, K. Genetic alterations in pancreatic cancer. *World J. Gastroenterol.* **2007**, *7*, 4423-4430.
46. Terhune, P.G.; Phifer, D.M.; Tosteson, T.D.; Longnecker, D.S. K-*ras* mutation infocal proliferative lesions of human pancreas. *Cancer Epidem. Biomark. Prev.* **1998**, *7*, 515-521.
47. Caldas, C.; Hahn, S.A.; da Costa, L.T.; Redston, M.S.; Schutte, M.; Seymour, A.B.; Weinstein, C.L.; Hruban, R.H.; Yeo, C.J.; Kern, S.E. Frequent somatic mutations and homozygous deletions of the p16 (MTS1) gene in pancreatic adenocarcinoma. *Nat. Genet.* **1994**, *8*, 27-32.
48. Hruban, R.H.; Offerhaus, G.J.A.; Kern, S.E.; Goggins, M.; Wilentz, R.E.; Yeo, C.J. Tumor-suppressor genes in pancreatic cancer. *J. Hepatobilary Pancreat. Surg.* **1998**, *5*, 383-391.

49. Li, J.; Weghorst, C.M.; Tsutsumi, M.; Poi, M.J.; Knobloch, T.J.; Casto, B.C.; Melvin, W.S.; Tsai, M.; Muscarella, P. Frequent *p16^{INK4A}/CDKN2A* alterations in chemically induced Syrian golden hamster pancreatic tumors. *Carcinogenesis* **2004**, *25*, 263-268.
50. Hahn, S.A.; Schutte, M.; Hoque, A.T.; Moskaluk, C.A.; da Costa, L.T.; Rozenblum, E.; Weinstein, C.L.; Fischer, A.; Yeo, C.J.; Hruban, R.H.; Kern, S.E. *DPC4*, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science* **1996**, *271*, 350-353.
51. Shimizu, K.; Kitahashi, T.; Fujii, H.; Tsutsumi, M.; Mori, T.; Honoki, K.; Tsujiuchi, T. Alterations in the *Smad4* gene in hamster pancreatic duct adenocarcinomas and established cell lines. *Oncol. Rep.* **2006**, *16*, 85-89.
52. Höhne, M.W.; Halatsch, M.E.; Kahl, G.F.; Weinel, R.J. Frequent loss of expression of the potential tumor suppressor gene *DCC* in ductal pancreatic adenocarcinoma. *Cancer Res.* **1992**, *52*, 2616-2619.
53. Chang, K.W.; Laconi, S.; Mangold, K.A.; Hubchak, S.; Scarpelli, D.G. Multiple genetic alterations in hamster pancreatic ductal adenocarcinomas. *Cancer Res.* **1995**, *55*, 2560-1568.
54. Barton, C.M.; Staddon, S.L.; Hughes, C.M.; Hall, P.A.; O'Sullivan, C.; Klöppel, G.; Theis, B.; Russell, R.C.; Neoptolemos, J.; Williamson, R.C.; Lane, D.P.; Lemoine, N.R. Abnormalities of the p53 tumour suppressor gene in human pancreatic cancer. *Br. J. Cancer* **1991**, *64*, 1076-1082.
55. Ruggeri, B.; Zhang, S.Y.; Caamano, J.; DiRado, M.; Flynn, S.D.; Klein-Szanto, A.J. Human pancreatic carcinomas and cell lines reveal frequent and multiple alterations in the *p53* and *Rb-1* tumor-suppressor genes. *Oncogene* **1992**, *7*, 1503-1511.
56. Rozenblum, E.; Schutte, M.; Goggins, M.; Hahn, S.A.; Panzer, S.; Zahurak, M.; Goodman, S.N.; Sohn, T.A.; Hruban, R.H.; Yeo, C.J.; Kern, S.E. Tumor-suppressive pathways in pancreatic carcinoma. *Cancer Res.* **1997**, *57*, 1731-1734.
57. Okita, S.; Tsutsumi, M.; Onji, M.; Konishi, Y. p53 mutation without allelic loss and absence of mdm-2 amplification in a transplantable hamster pancreatic ductal adenocarcinoma and derived cell lines but not primary ductal adenocarcinomas in hamsters. *Mol. Carcinog.* **1995**, *13*, 266-271.
58. Sorio, C.; Baron, A.; Orlandini, S.; Zamboni, G.; Pederzoli, P.; Huebner, K.; Scarpa, A. The *FHIT* gene is expressed in pancreatic ductular dells and is altered in pancreatic cancers. *Cancer Res.* **1999**, *59*, 1308-1314.
59. Gopalakrishnan, V.K.; Banerjee, A.G.; Vishwanatha, J.K. Effect of *FHIT* gene replacement on growth, cell cycle and apoptosis in pancreatic cancer cells. *Pancreatol.* **2003**, *3*, 293-302.
60. Tsijiuchi, T.; Sasaki, Y.; Kubozoe, T.; Konishi, Y.; Tsutsumi, M. Alterations in the Fhit gene in pancreatic duct adenocarcinomas induced by N-nitrosobis(2-oxopropyl)amine in hamsters. *Mol. Carcinog.* **2003**, *36*, 60-66.
61. Kobitsu, K.; Tsutsumi, M.; Tsujiuchi, T.; Suzuki, F.; Kido, A.; Okajima, E.; Fukuda, T.; Sakaki, T.; Konishi, Y. Shortened telomere length and increased telomerase activity in hamster pancreatic duct adeenocarcinomas and cell line. *Mol. Carcinog.* **1997**, *18*, 153-159.
62. Hiyama, E.; Kodama, T.; Shinbara, K.; Iwao, T.; Itoh, M.; Hiyama, K.; Shay, J.W.; Matsuura, Y.; Yokoyama, T. Telomerase activity is detected in pancreatic cancer but not in benign tumors. *Cancer Res.* **1997**, *57*, 326-331.
63. Tsutsumi, M.; Kadomatsu, K.; Tsujiuchi, T.; Sakitani, H.; Ikematsu, S.; Kubozoe, T.; Yoshimoto, M.; Marumatsu, T.; Sakuma, S.; Konishi, Y. Overexpression of midkine in pancreatic duct

adenocarcinomas induced by N-nitrosobis(2-oxopropyl)amine in hamsters and their cell line. *Jpn. J. Cancer Res.* **2000**, *91*, 979-986.

64. Tsutsui, J.; Kadomatsu, K.; Matsubara, S.; Nakagawara, A.; Hamanoue, M.; Kakao, S.; Shimazu, H.; Ohi, Y.; Muramatsu, T. A new family of heparin-binding growth / differentiation factors: Increased midkine expression in Wilms' tumor and other human carcinomas. *Cancer Res.* **1993**, *53*, 1281-1285.
65. Crowell, P.L.; Schmidt, C.M.; Yip-Schneider, M.T.; Savage, J.J.; Hertzler, D.A. 2nd; Cummings, W.O. Cyclooxygenase-2 expression in hamster and human pancreatic neoplasia. *Neoplasia* **2006**, *8*, 437-445.
66. Iki, K.; Tsutsumi, M.; Kido, A.; Sakitani, H.; Takahama, M.; Yoshimoto, M.; Motoyama, M.; Tatsumi, K.; Tsunoda, T.; Konishi, Y. Expression of matrix metalloproteinase 2 (MMP-2), membrane-type 1 MMP and tissue inhibitor of metalloproteinase 2 and activation of proMMP-2 in pancreatic duct adenocarcinomas in hamsters treated with N-nitrosobis(2-oxopropyl)amine. *Carcinogenesis* **1999**, *20*, 1323-1329.
67. Määttä, M.; Soini, Y.; Liakka, A.; Autio-Harmainen, H. Differential expression of matrix metalloproteinase (MMP)-2, MMP-9, and membrane type 1-MMP in hepatocellular and pancreatic adenocarcinoma: Implications for tumor progression and clinical prognosis. *Clin. Cancer Res.* **2000**, *6*, 2726-2734.
68. Michaud, D.S. Epidemiology of pancreatic cancer. *Minerva Chir.* **2004**, *59*, 99-111.
69. Birt, D.F.; Salmasi, S.; Pour, P.M. Enhancement of experimental pancreatic cancer in Syrian golden hamsters by dietary fat. *J. Natl. Cancer Inst.* **1981**, *67*, 1327-1332.
70. Birt, D.F.; Julius, A.D.; Dwork, E.; Hanna, T.; White, L.T.; Pour, P.M. Comparison of the effects of dietary beef tallow and corn oil on pancreatic carcinogenesis in the hamster model. *Carcinogenesis* **1990**, *11*, 745-748.
71. Heukamp, I.; Kilian, M.; Gregor, J.I.; Kiewert, C.; Schimke, I.; Kristiansen, G.; Walz, M.K.; Jacobi, C.A.; Wenger, F.A. Impact of polyunsaturated fatty acids on hepato-pancreatic prostaglandin and leukotriene concentration in ductal pancreatic cancer – Is there a correlation to tumor growth and liver metastasis? *Prostaglandins Leukot. Essent. Fatty Acids* **2006**, *74*, 223-233.
72. Khasawneh, J.; Schulz, M.D.; Walch, A.; Rozman, J.; Hrabe de Angelis, M.; Klingenspor, M.; Buck, A.; Schwaiger, M; Saur, D.; Schmid, R.M.; Klöppel, G.; Sipos, B.; Greten, F.R.; Arkan, M.C. Inflammation and mitochondrial fatty acid beta-oxidation link obesity to early tumor promotion. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 3354-3359.
73. Strouch, M.J.; Ding, Y.; Salabat, M.R.; Melstrom, L.G.; Adrian, K.; Quinn, C.; Pelham, C.; Rao, S.; Adrian, T.E.; Bentrem, D.J.; Grippo, P.J. A high omega-3 fatty acid diet mitigates murine pancreatic precancer development. *J. Surg. Res.* **2011**, *165*, 77-81.
74. Boutros, C.; Somasundar, P.; Razzak, A.; Helton, S.; Espat, N.J. Omega-3 fatty acids: Investigation from cytokine regulation to pancreatic cancer gene suppression. *Arch. Surg.* **2010**, *145*, 515-520.
75. Pour, P.M.; Patil, K. Modification of pancreatic carcinogenesis in the hamster model. X. Effect of streptozotocin. *J. Natl. Cancer Inst.* **1983**, *71*, 1059-1065.
76. Bell, R.H., Jr.; Strayer, D.S. Streptozotocin prevents development of nitrosamine-induced pancreatic cancer in the Syrian hamster. *J. Surg. Oncol.* **1983**, *24*, 258-262.

77. Bell, R.H., Jr.; McCullough, P.J.; Pour, P.M. Influence of diabetes on susceptibility to experimental pancreatic cancer. *Am. J. Surg.* **1988**, *155*, 159-164.
78. Fisher, W.E.; McCullough, P.J.; Ray, M.B.; Rogers, D.H.; Bell, R.H., Jr. Diabetes enhances growth of pancreatic carcinoma cells. *Surgery* **1988**, *104*, 431-436.
79. Zyromski, N.J.; Mathur, A.; Pitt, H.A.; Wade, T.E.; Wang, S.; Nakshatri, P.; Swartz-Basile, D.A.; Nakshatri, H. Obesity potentiates the growth and dissemination of pancreatic cancer. *Surgery* **2009**, *146*, 258-263.
80. White, P.B.; True, E.M.; Ziegler, K.M.; Wang, S.S.; Swartz-Basile, D.A.; Pitt, H.A.; Zyromski, N.J. Insulin, Leptin, and tumoral adipocytes promote murine pancreatic cancer growth. *J. Gastrointest. Surg.* **2010**, *14*, 1888-1893.
81. Satake, K.; Mukai, R.; Kato, Y.; Umeyama, K. Effects of cerulein on the normal pancreas and on experimental pancreatic carcinoma in the Syrian golden hamster. *Pancreas* **1986**, *1*, 246-253.
82. Pour, P.M.; Takahashi, M.; Donnelly, T.; Stepan, K. Modification of pancreatic carcinogenesis in the hamster model. IX. Effect of pancreatitis. *J. Natl. Cancer Inst.* **1983**, *71*, 607-613.
83. Otsuki, M.; Tashiro, M. 4. Chronic pancreatitis and pancreatic cancer, lifestyle-related diseases. *Intern. Med.* **2007**, *46*, 109-113.
84. Go, V.L.W.; Gukovskaya, A.; Pandol, S.J. Alcohol and pancreatic cancer. *Alcohol* **2005**, *35*, 205-211.
85. Wendt, L.R.; Osvaldt, A.B.; Bersch, V.P.; Schumacher, R. de C.; Edelweiss, M.I.; Rohde, L. Pancreatic intraepithelial neoplasia and ductal adenocarcinoma induced by DMBA in mice: Effects of alcohol and caffeine. *Acta Cir. Bras.* **2007**, *22*, 202-209.
86. Bersch, V.P.; Osvaldt, A.B.; Edelweiss, M.I.; Schumacher, R. de C.; Wendt, L.R.; Abreu, L.P.; Blom, C.B.; Abreu, G.P.; Costa, L.; Piccinini, P.; Rohde, L. Effect of nicotine and cigarette smoke on an experimental model of intraepithelial lesions and pancreatic adenocarcinoma induced by 7,12-dimethylbenzanthracene in mice. *Pancreas* **2009**, *38*, 65-70.
87. Mizumoto, K.; Tsutsumi, M.; Denda, A.; Konishi, Y. Rapid production of pancreatic carcinoma by initiation with N-nitroso-bis(2-oxopropyl)amine and repeated augmentation pressure in hamsters. *J. Natl. Cancer Inst.* **1988**, *80*, 1564-1567.
88. World Cancer Research Fund/American Institute for Cancer Research. *Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective*; AICR: Washington, DC, USA, 2007.
89. Hart, A.R.; Kennedy, H.; Harvey, I. Pancreatic cancer: A review of the evidence on causation. *Clin. Gastroenterol. Hepatol.* **2008**, *6*, 275-282.
90. Anderson, K.E.; Sinha, R.; Kulldorff, M.; Gross, M.; Lang, N.P.; Barber, C.; Harnack, L.; DiMagno, E.; Bliss, R.; Kadlubar, F.F. Meat intake and cooking techniques: Associations with pancreatic cancer. *Mutat. Res.* **2002**, *506-507*, 225-231.
91. Yoshimoto, M.; Tsutsumi, M.; Iki, K.; Sasaki, Y.; Tsujiuchi, T.; Sugimura, T.; Wakabayashi, K.; Konishi, Y. Carcinogenicity of heterocyclic amines for the pancreatic duct epithelium in hamsters. *Cancer Lett.* **1999**, *143*, 235-239.
92. Li, D.; Day, R.S.; Bondy, M.L.; Shinha, R.; Tguyen, N.T.; Evans, D.B.; Abbruzzese, J.L.; Hassan, M.M. Dietary mutagen exposure and risk of pancreatic cancer. *Cancer Epidemiol. Biomarkers Prev.* **2007**, *16*, 655-661.

93. Lin, Y.; Kikuchi, S.; Tamakoshi, A.; Yagyu, K.; Obata, Y.; Inaba, Y.; Kurosawa, M.; Kawamura, T.; Motohashi, Y.; Ishibashi, T. Dietary habits and pancreatic cancer risk in a cohort of middle-aged and elderly Japanese. *Nutr. Cancer* **2006**, *56*, 40-49.
94. Bravi, F.; Polesel, J.; Bosetti, C.; Talamini, R.; Negri, E.; Dal Maso, L.; Serraino, D.; La Vecchia, C. Dietary intake of selected micronutrients and the risk of pancreatic cancer: An Italian case-control study. *Ann. Oncol.* **2010**, *22*, 202-206.
95. Wenger, F.A.; Kilian, M.; Ridders, J.; Stahlknecht, P.; Schimke, I.; Guski, H.; Jacobi, C.A.; Müller, J.M. Influence of antioxidative vitamins A, C and E on lipid peroxidation in BOP-induced pancreatic cancer in Syrian hamsters. *Prostaglandins Leukot. Essent. Fatty Acids* **2001**, *65*, 165-171.
96. Wolff, R.A. Chemoprevention for pancreatic cancer. *Int. J. Gastrointest Cancer* **2003**, *33*, 27-41.
97. Kusama, T.; Mukai, M.; Iwasaki, T.; Tatsuta, M.; Matsumoto, Y.; Akedo, H.; Inoue, M.; Nakamura, H. 3-Hydroxy-3-methylglutaryl-coenzyme a reductase inhibitors reduce human pancreatic cancer cell invasion and metastasis. *Gastroenterology* **2002**, *122*, 308-317.
98. Khurana, V.; Sheth, A.; Caldito, G.; Barkin, J.S. Statins reduce the risk of pancreatic cancer in humans: A case-control study of half a million veterans. *Pancreas* **2007**, *34*, 260-265.
99. Bonovas, S.; Filioussi, K.; Sitaras, N.M. Statins are not associated with a reduced risk of pancreatic cancer at the population level, when taken at low doses for managing hypercholesterolemia: Evidence from a meta-analysis of 12 studies. *Am. J. Gastroenterol.* **2008**, *103*, 2646-2651.
100. Bosetti, C.; La Vecchia, C. Aspirin and cancer risk: A summary review to 2007. *Recent Results Cancer Res.* **2009**, *181*, 231-251.
101. Larsson, S.C.; Giovannucci, E.; Bergkvist, L.; Wolk, A. Aspirin and nonsteroidal anti-inflammatory drug use and risk of pancreatic cancer: A meta-analysis. *Cancer Epidemiol. Biomarkers Prev.* **2006**, *15*, 2561-2564.
102. Bonifazi, M.; Gallus, S.; Bosetti, C.; Polesel, J.; Serraino, D.; Talamini, R.; Negi, E.; La Vecchia, C. Aspirin use and pancreatic cancer risk. *Eur. J. Cancer Prev.* **2010**, *19*, 352-354.
103. Anderson, K.E.; Johnson, T.W.; Lazovich, D.; Folsom, A.R. Association between nonsteroidal anti-inflammatory drug use and the incidence of pancreatic cancer. *J. Natl. Cancer Inst.* **2002**, *94*, 1168-1171.
104. Schernhammer, E.S.; Kang, J.H.; Chan, A.T.; Michaud, D.S.; Skinner, H.G.; Giovannucci, E.; Colditz, G.A.; Fuchs, C.S. A prospective study of aspirin use and the risk of pancreatic cancer in women. *J. Natl. Cancer Inst.* **2004**, *96*, 22-28.
105. Kisfalvi, K.; Eibl, G.; Sinnett-Smith, J.; Rozengurt, E. Metformin disrupts crosstalk between G protein-coupled receptor and insulin receptor signaling systems and inhibits pancreatic cancer growth. *Cancer Res.* **2009**, *69*, 6539-6545.
106. Feng, Y.H.; Velazquez-Torres, G.; Gully, C.; Chen, J.; Lee, M.H.; Yeung, S.C. The impact of type 2 diabetes and antidiabetic drugs on cancer cell growth. *Cell. Mol. Med.* **2010**, doi: 10.1111/j.1582-4934.2010.01083.x.
107. Li, D.; Yeung, S.C.; Hassan, M.M.; Konopleva, M.; Abbruzzese, J.L. Antidiabetic therapies affect risk of pancreatic cancer. *Gastroenterology* **2009**, *137*, 482-488.

108. Lin, Y.; Tamakoshi, A.; Hayakawa, T.; Naruse, S.; Kitagawa, M.; Ohno, Y. Nutritional factors and risk of pancreatic cancer: A population-based case-control study based on direct interview in Japan. *J. Gastroenterol.* **2005**, *40*, 297-301.
109. Takeuchi, T.; Nakao, M.; Nomura, K.; Yano, E. Association of metabolic syndrome with smoking and alcohol intake in Japanese men. *Nicotine Tob. Res.* **2009**, *11*, 1093-1098.
110. Ashakumary, L.; Vijayammal, P.L. Effect of nicotine on lipoprotein metabolism in rats. *Lipids* **1997**, *32*, 311-315.
111. Hill, M.J.; Metcalfe, D.; McTernan, P.G. Obesity and diabetes: Lipids, 'nowhere to run to'. *Clin. Sci. (Lond)* **2009**, *116*, 113-123.
112. Hillon, P.; Guiu, B.; Vincent, J.; Petit, J.M. Obesity, type 2 diabetes and risk of digestive cancer. *Gastroenterol. Clin. Biol.* **2010**, *34*, 529-533.
113. Takeuchi, Y.; Takahashi, M.; Sakano, K.; Mutoh, M.; Niho, N.; Yamamoto, M.; Sato, H.; Sugimura, T.; Wakabayashi, K. Suppression of *N*-nitrosobis(2-oxopropyl)amine-induced pancreatic carcinogenesis in hamsters by pioglitazone, a ligand of peroxisome proliferator-activated receptor gamma. *Carcinogenesis* **2007**, *28*, 1692-1696.
114. Schneider, M.B.; Matsuzaki, H.; Haorah, J.; Ulrich, A.; Standop, J.; Ding, X.Z.; Adrian, T.E.; Pour, P.M. Prevention of pancreatic cancer induction in hamsters by metformin. *Gastroenterology* **2001**, *120*, 1263-1270.
115. Staels, B. Metformin and pioglitazone: Effectively treating insulin resistance. *Curr. Med. Res. Opin.* **2006**, *22* (Suppl. 2), S27-S37.
116. Tucker, O.N.; Dannenberg, A.J.; Yang, E.K.; Zhang, F.; Teng, L.;, Daly, J.M.; Soslow, R.A.; Masferrer, J.L.; Woerner, B.M.; Koki, A.T.; Fahey, T.J. 3rd. Cyclooxygenase-2 expression is up-regulated in human pancreatic cancer. *Cancer Res.* **1999**, *59*, 987-990.
117. Molina, M.A.; Sitja-Arnau, M.; Lemoine, M.G.; Frazier, M.L.; Sinicrope, F.A. Increased cyclooxygenase-2 expression in human pancreatic carcinomas and cell lines: Growth inhibition by nonsteroidal anti-inflammatory drugs. *Cancer Res.* **1999**, *59*, 4356-4362.
118. Furukawa, F.; Nishikawa, A.; Lee, I.S.; Kanki, K.; Umemura, T.; Okazaki, K.; Kawamori, T.; Wakabayashi, K.; Hirose, M. A cyclooxygenase-2 inhibitor, nimesulide, inhibits postinitiation phase of N-nitrosobis(2-oxopropyl)amine-induced pancreatic carcinogenesis in hamsters. *Int. J. Cancer* **2003**, *104*, 269-273.
119. Takahashi, M.; Furukawa, F.; Toyoda, K.; Sato, H.; Hasegawa, R.; Imaida, K.; Hayashi, Y. Effects of various prostaglandin synthesis inhibitors on pancreatic carcinogenesis in hamsters after initiation with N-nitrosobis(2-oxopropyl)amine. *Carcinogenesis* **1990**, *11*, 393-395.
120. Nishikawa, A.; Furukawa, F.; Lee, I.S.; Tanaka, T.; Hirose, M. Potent chemopreventive agents against pancreatic cancer. *Curr. Cancer Drug Targets* **2004**, *4*, 373-384.
121. Ouyang, N.; Williams, J.L.; Tsioulias, G.J.; Gao, J.; Iatropoulos, M.J.; Kopelovich, L.; Kashfi, K.; Rigas, B. Nitric oxide-donating aspirin prevents pancreatic cancer in a hamster tumor model. *Cancer Res.* **2006**, *66*, 4503-4511.
122. Schuller, H.M.; Zhang, L.; Weddle, D.L.; Castonguay, A.; Walker, K.; Miller, M.S. The cyclooxygenase inhibitor ibuprofen and the FLAP inhibitor MK886 inhibit pancreatic carcinogenesis induced in hamsters by transplacental exposure to ethanol and the tobacco carcinogen NNK. *J. Cancer Res. Clin. Oncol.* **2002**, *128*, 525-532.

123. Fendrich, V.; Chen, N.M.; Neef, M.; Waldmann, J.; Buchholz, M.; Feldmann, G.; Slater, E.P.; Maitra, A.; Bartsch, D.K. The angiotensin-I-converting enzyme inhibitor enalapril and aspirin delay progression of pancreatic intraepithelial neoplasia and cancer formation in a genetically engineered mouse model of pancreatic cancer. *Gut* **2010**, *59*, 630-637.
124. Funahashi, H.; Satake, M.; Dawson, D.; Huynh, N.-A.; Reber, H.A.; Hines, O.J.; Eibl, G. Delayed progression of pancreatic intraepithelial neoplasia in a conditional $Kras^{G12D}$ mouse model by a selective cyclooxygenase-2 inhibitor. *Cancer Res.* **2007**, *67*, 7068-7071.
125. Hennig, R.; Ding, X.Z.; Tong, W.G.; Schneider, M.B.; Standop, J.; Friess, H.; Büchler, M.W.; Pour, P.M.; Adrian, T.E. 5-Lipoxygenase and leukotriene B(4) receptor are expressed in human pancreatic cancers but not in pancreatic ducts in normal tissue. *Am. J. Pathol.* **2002**, *161*, 421-428.
126. Hennig, R.; Grippo, P.; Ding, X.Z.; Rao, S.M.; Buchler, M.W.; Friess, H.; Talamonti, M.S.; Bell, R.H.; Adrian, T.E. 5-Lipoxygenase, a marker for early pancreatic intraepithelial neoplastic lesions. *Cancer Res.* **2005**, *65*, 6011-6016.
127. Wenger, F.A.; Kilian, M.; Bisevac, M.; Khodadayan, C.; von Seebach, M.; Schimke, I.; Guski, H.; Müller, J.M. Effects of Celebrex and Zyflo on liver metastasis and lipidperoxidation in pancreatic cancer in Syrian hamsters. *Clin. Exp. Metastasis* **2002**, *19*, 681-687.
128. Vickers, S.M.; MacMillan-Crow, L.A.; Green, M.; Ellis, C.; Thompson, J.A. Association of increased immunostaining for inducible nitric oxide synthase and nitrotyrosine with fibroblast growth factor transformation in pancreatic cancer. *Arch. Surg.* **1999**, *134*, 245-251.
129. Kong, G.; Kim, E.K.; Kim, W.S.; Lee, K.T.; Lee, Y.W.; Lee, J.K.; Paik, S.W.; Rhee, J.C. Role of cyclooxygenase-2 and inducible nitric oxide synthase in pancreatic cancer. *J. Gastroenterol. Hepatol.* **2002**, *17*, 914-921.
130. Franco, L.; Doria, D.; Bertazzoni, E.; Benini, A.; Bassi, C. Increased expression of inducible nitric oxide synthase and cyclooxygenase-2 in pancreatic cancer. *Prostaglandins Other Lipid Mediat.* **2004**, *73*, 51-58.
131. Takahashi, M.; Kitahashi, T.; Ishigamori, R.; Mutoh, M.; Komiya, M.; Sato, H.; Kamanaka, Y.; Naka, M.; Maruyama, T.; Sugimura, T.; Wakabayashi, K. Increased expression of inducible nitric oxide synthase (iNOS) in *N*-nitrosobis(2-oxopropyl)amine-induced hamster pancreatic carcinogenesis and prevention of cancer development by ONO-1714, an iNOS inhibitor. *Carcinogenesis* **2008**, *29*, 1608-1613.
132. Takahashi, M.; Mutoh, M.; Shoji, Y.; Kamanaka, Y.; Naka, M.; Maruyama, T.; Sugimura, T.; Wakabayashi, K. Transfection of K-ras^{Asp12} cDNA markedly elevates IL-1beta- and lipopolysaccharide-mediated inducible nitric oxide synthase expression in rat intestinal epithelial cells. *Oncogene* **2003**, *22*, 7667-7676.
133. Koshiba, T.; Hosotani, R.; Wada, M.; Miyamoto, Y.; Fujimoto, K.; Lee, J.U.; Doi, R.; Arii, S.; Imamura, M. Involvement of matrix metalloproteinase-2 activity in invasion and metastasis of pancreatic carcinoma. *Cancer* **1998**, *82*, 642-650.
134. Kilian, M.; Gregor, J.I.; Heukamp, I.; Hanel, M.; Ahlgrimm, M.; Schimke, I.; Kristiansen, G.; Ommer, A.; Walz, M.K.; Jacobi, C.A.; Wenger, F.A. Matrix metalloproteinase inhibitor RO 28-2653 decreases liver metastasis by reduction of MMP-2 and MMP-9 concentration in BOP-induced ductal pancreatic cancer in Syrian Hamsters: Inhibition of matrix metalloproteinases in pancreatic cancer. *Prostaglandins Leukot. Essent. Fatty Acids* **2006**, *75*, 429-434.

135. Nakamura, H.; Nishikawa, A.; Furukawa, F.; Kasahara, K.; Miyauchi, M.; Son, H.Y.; Hirose, M. Inhibitory effects of protocatechuic acid on the post-initiation phase of hamster pancreatic carcinogenesis induced by *N*-nitrosobis(2-oxopropyl)amine. *Anticancer Res.* **2000**, *20*, 3423-3427.
136. Hiura, A.; Tsutsumi, M.; Satake, K. Inhibitory effect of green tea extract on the process of pancreatic carcinogenesis induced by *N*-nitrosobis-(2-oxypropyl)amine (BOP) and on tumor promotion after transplantation of *N*-nitrosobis-(2-hydroxypropyl)amine (BHP)-induced pancreatic cancer in Syrian hamsters. *Pancreas* **1997**, *15*, 272-277.
137. Mizumoto, K.; Ito, S.; Kitazawa, S.; Tsutsumi, M.; Denda, A.; Konishi, Y. Inhibitory effect of butylated hydroxyanisole administration on pancreatic carcinogenesis in Syrian hamsters initiated with *N*-nitrosobis(2-oxopropyl)amine. *Carcinogenesis* **1989**, *10*, 1491-1494.
138. Yokomatsu, H.; Hiura, A.; Tsutsumi, M.; Satake, K. Inhibitory effect of sarcophytol A on pancreatic carcinogenesis after initiation by *N*-nitrosobis(2-oxypropyl)amine in Syrian hamsters. *Pancreas* **1996**, *13*, 154-159.
139. Furukawa, F.; Nishikawa, A.; Lee, I.S.; Son, H.Y.; Nakamura, H.; Miyauchi, M.; Takahashi, M.; Hirose, M. Inhibition by methionine of pancreatic carcinogenesis in hamsters after initiation with *N*-nitrosobis(2-oxopropyl) amine. *Cancer Lett.* **2000**, *152*, 163-167.
140. Nishikawa, A.; Furukawa, F.; Uneyama, C.; Ikezaki, S.; Tanakamaru, Z.; Chung, F.L.; Takahashi, M.; Hayashi, Y. Chemopreventive effects of phenethyl isothiocyanate on lung and pancreatic tumorigenesis in *N*-nitrosobis(2-oxopropyl)amine-treated hamsters. *Carcinogenesis* **1996**, *17*, 1381-1384.
141. Nishikawa, A.; Lee, I.S.; Uneyama, C.; Furukawa, F.; Kim, H.C.; Kasahara, K.; Huh, N.; Takahashi, M. Mechanistic insights into chemopreventive effects of phenethyl isothiocyanate in *N*-nitrosobis(2-oxopropyl)amine-treated hamsters. *Jpn. J. Cancer Res.* **1997**, *88*, 1137-1142.
142. Nishikawa, A.; Furukawa, F.; Kasahara, K.; Tanakamaru, Z.; Miyauchi, M.; Nakamura, H.; Ikeda, T.; Imazawa, T.; Hirose, M. Failure of phenethyl isothiocyanate to inhibit hamster tumorigenesis induced by N-nitrosobis(2-oxopropyl)amine when given during the post-initiation phase. *Cancer Lett.* **1999**, *141*, 109-115.
143. Nishikawa, A.; Furukawa, F.; Ikezaki, S.; Tanakamaru, Z.Y.; Chung, F.L.; Takahashi, M.; Hayashi, Y. Chemopreventive effects of 3-phenylpropyl isothiocyanate on hamster lung tumorigenesis initiated with *N*-nitrosobis(2-oxopropyl)amine. *Jpn. J. Cancer Res.* **1996**, *87*, 122-126.
144. Son, H.Y.; Nishikawa, A.; Furukawa, F.; Lee, I.S.; Ikeda, T.; Miyauchi, M.; Nakamura, H.; Hirose, M. Modifying effects of 4-phenylbutyl isothiocyanate on *N*-nitrosobis(2-oxopropyl)amine-induced tumorigenesis in hamsters. *Cancer Lett.* **2000**, *160*, 141-147.
145. Kuroiwa, Y.; Nishikawa, A.; Kitamura, Y.; Kanki, K.; Ishii, Y.; Umemura, T.; Hirose, M. Protective effects of benzyl isothiocyanate and sulforaphane but not resveratrol against initiation of pancreatic carcinogenesis in hamsters. *Cancer Lett.* **2006**, *241*, 275-280.
146. Furukawa, F.; Nishikawa, A.; Chihara, T.; Shimpo, K.; Beppu, H.; Kuzuya, H.; Lee, I.S.; Hirose, M. Chemopreventive effects of Aloe arborescens on N-nitrosobis(2-oxopropyl)amine-induced pancreatic carcinogenesis in hamsters. *Cancer Lett.* **2002**, *178*, 117-122.
147. Clapper, M.L.; Wood, M.; Leahy, K.; Lang, D.; Miknyoczki, S.; Ruggeri, B.A. Chemopreventive activity of Oltipraz against *N*-nitrosobis(2-oxopropyl)amine (BOP)-induced ductal pancreatic

carcinoma development and effects on survival of Syrian golden hamsters. *Carcinogenesis* **1995**, *16*, 2159-2165.

148. Askari, M.D.; Tsao, M.S.; Schuller, H.M. The tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone stimulates proliferation of immortalized human pancreatic duct epithelia through beta-adrenergic transactivation of EGF receptors. *J. Cancer Res. Clin. Oncol.* **2005**, *131*, 639-648.
149. Schuller, H.M.; Al-Wadei, H.A. Neurotransmitter receptors as central regulators of pancreatic cancer. *Future Oncol.* **2010**, *6*, 221-228.
150. Al-Wadei, H.A.; Al-Wadei, M.H.; Schuller, H.M. Prevention of pancreatic cancer by the beta-blocker propranolol. *Anticancer Drugs.* **2009**, *20*, 477-482.
151. Arafat, H.A.; Gong, Q.; Chipitsyna, G.; Rizvi, A.; Saa, C.T.; Yeo, C.J. Antihypertensives as novel antineoplastics: Angiotensin-I-converting enzyme inhibitors and angiotensin II type 1 receptor blockers in pancreatic ductal adenocarcinoma. *J. Am. Coll. Surg.* **2007**, *204*, 996-1005
152. Mohammed, A.; Janakiram, N.B.; Li, Q.; Madka, V.; Ely, M.; Lightfoot, S.; Crawford, H.; Steele, V.E.; Rao, C.V. The epidermal growth factor receptor inhibitor gefitinib prevents the progression of pancreatic lesions to carcinoma in a conditional LSL-Kras$^{G12D/+}$ transgenic mouse model. *Cancer Prev. Res. (Phila)* **2010**, *3*, 1417-1426.
153. Morton, J.P.; Karim, S.A.; Graham, K.; Timpson, P.; Jamieson, N.; Athineos, D.; Doyle, B.; McKay, C.; Heung, M.Y.; Oien, K.A.; Frame, M.C.; Evans, T.R.; Sansom, O.J.; Brunton, V.G. Dasatinib inhibits the development of metastases in a mouse model of pancreatic ductal adenocarcinoma. *Gastroenterology* **2010**, *139*, 292-303.
154. Liby, K.T.; Royce, D.B.; Risingsong, R.; Williams, C.R.; Maitra, A.; Hruban, R.H.; Sporn, M.B. Synthetic triterpenoids prolong survival in a transgenic mouse model of pancreatic cancer. *Cancer Prev. Res.* **2010**, *3*, 1427-1434.
155. Grippo, P.J.; Tuveson, D.A. Deploying mouse models of pancreatic cancer for chemoprevention studies. *Cancer Prev. Res.* **2010**, *3*, 1382-1387.

Published OnlineFirst January 13, 2011; DOI:10.1158/1940-6207.CAPR-10-0028

Research Article

Cancer Prevention Research

Inhibition of Intestinal Polyp Formation by Pitavastatin, a HMG-CoA Reductase Inhibitor

Naoya Teraoka[1], Michihiro Mutoh[1], Shinji Takasu[1], Toshiya Ueno[1], Masafumi Yamamoto[1], Takashi Sugimura[1], and Keiji Wakabayashi[1,2]

Abstract

It has been suggested that hyperlipidemia is positively associated with colon carcinogenesis. Statins, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors, reduce serum lipid levels. In this study, we clarified the effects of a novel chemically synthesized statin, pitavastatin, on intestinal polyp formation in Min mice, and further examined serum lipid and adipocytokine levels, and proinflammatory and adipocytokine gene levels in intestinal mucosa of Min mice. Treatment with pitavastatin at doses of 20 and 40 ppm decreased the total number of polyps dose-dependently to 85.2% and 65.8% ($P < 0.05$) of the untreated value, respectively. Serum levels of total cholesterol and triglyceride were slightly reduced and those of IL-6, leptin, and MCP-1 were decreased by 40-ppm pitavastatin treatment. mRNA expression levels of *cyclooxygenase-2*, *IL-6*, *inducible nitric oxide* (*iNOS*), *MCP-1*, and *Pai-1* were significantly reduced in intestinal nonpolyp parts by pitavastatin treatment. Among them, *iNOS* mRNA levels were also reduced in the intestinal polyps. Moreover, oxidative stress represented by 8-nitroguanosine in the small intestinal epithelial cells was reduced by pitavastatin treatment. Related to these proinflammatory genes, PPARγ activity was activated in the intestinal nonpolyp parts and in the liver of Min mice with pitavastatin treatment. These results indicated that pitavastatin has potential benefit for the suppression of intestinal polyp development. *Cancer Prev Res; 4(3); 445–53. ©2011 AACR.*

Introduction

Epidemiological studies have suggested that mortality and morbidity of colon cancer are increasing in developed countries (1, 2). Thus, it is very important to establish effective methods to prevent colon cancer development. Consumption of a high-fat diet is a considerable risk factor of colon cancer with clear link with hyperlipidemia. Hyperlipidemia has also been indicated to be positively associated with colon carcinogenesis (3, 4). We have reported *Apc* deficient, Min and Apc^{1309} mice, which developed a large number of intestinal polyps showed the hyperlipidemic state (5–7). Interestingly, improvement of hyperlipidemic state by peroxisome proliferator-activated receptor (PPAR) α and γ agonists and a selective LPL-inducing agent, NO-1886, which does not possess PPARs agonistic activity, suppressed intestinal polyp formation in Min mice. Thus, it is conceivable that drugs, which effectively improve hyperlipidemia, could also prevent colon cancer development.

Authors' Affiliations: [1]Cancer Prevention Basic Research Project, National Cancer Center Research Institute, 5–1-1 Tsukiji, Chuo-ku, Tokyo, Japan; [2]School of Food and Nutritional Sciences, University of Shizuoka, 52–1 Yada, Suruga-ku, Shizuoka, Japan

Corresponding Author: Keiji Wakabayashi, School of Food and Nutritional Sciences, University of Shizuoka, 52-1 Yada, Surugaku, Shizuoka, Japan. Phone: 81-54-264-5835; E-mail: gp1576@u-shizuoka-ken.ac.jp

doi: 10.1158/1940-6207.CAPR-10-0028

Statins, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, are commonly used for the treatment of hypercholesterolemia (8, 9). Among statins, pravastatin, lovastatin, and simvastatin are so called the first-generation statins (10, 11), and fluvastatin is the second-generation statin (12). Recently, the third-generation statins, such as atorvastatin and rosuvastatin, were developed (13, 14), which strongly suppress serum LDL-cholesterol levels compared with the former generation statins. The Molecular Epidemiology of Colorectal Cancer (MECC) study has indicated that use of statins for 5 years or longer significantly reduced the risk of colorectal cancer by 47% (15). Meanwhile, some epidemiological studies using the first-generation statins, lovastatin and pravastatin (16, 17), are not fully consistent with above data. One of the reasons might be the suboptimal administration of the drug, in which efficacy of statins could not be shown, and another reason might be the difference of statin generation. Thus, epidemiological and/or experimental data on third-generation statins are desired to evaluate its chemopreventive effects on colon cancer.

Previous animal studies have shown that pravastatin and atorvastatin suppressed 1,2-dimethylhydrazine (DMH) or azoxymethane (AOM)-induced colon cancer development in mice and rats, respectively (18, 19). Recent animal studies have shown that 100-ppm atorvastatin reduced the incidnce of small intestinal polyp (adenoma) in Min mice to about 70% of the control group (20). Meanwhile, 10-ppm pitavastatin, a novel third-generation lipophilic

Published OnlineFirst January 13, 2011; DOI:10.1158/1940-6207.CAPR-10-0028

statin (21), reduced the incidence of colon adenoma or adenocarcinoma induced by AOM/dextran sodium sulfate treatment in ICR mice to about 78% of the control group (22). These results implied that pitavastatin may have a potent effect against colon tumor formation in rodent.

Pitavastatin may also have several clinical advantages over other statins. Similar serum triglyceride (TG) levels could be achieved by doses of pitavastatin (2 mg/d) lower than those of atorvastatin or rosuvastatin (10 mg/d). Lowering potentiality of pitavastatin on serum LDL-cholesterol is greater than that of pravastatin and is similar to atorvastatin (23, 24). As pitavastatin is hardly metabolized by cytochrome P450 compared with other statins, pitavastatin has advantage of not having unexpected interactions with other drugs.

In addition to the main function of statins, which is inhibition of the synthesis of mevalonate, statin also suppresses inflammation. Statins concomitantly suppress geranylgeranylation of protein, such as the small GTP-binding proteins RhoA, Ras, Cdc42, and Rac (25,26), which activate intracellular signaling molecules. Thus, pleiotropic action of pitavastatin could be involved in the suppression of cancer development.

In this study, we clarified the suppressive effect of pitavastatin on intestinal polyp development in Min mice. The mechanism involved in the suppressive effect of pitavastatin treatment on intestinal polyp formation in Min mice was also examined and further discussed.

Materials and Methods

Animals and chemicals

Male C57BL/6J-*Apc*$^{Min/+}$ mice (Min mice) were purchased from The Jackson Laboratory at 6 weeks of age and genotyped as previously reported (27). Heterozygotes of the Min strain and wild-type (C57BL/6J) mice were acclimated to laboratory conditions for 1 week. Four or five mice were housed per plastic cage with sterilized softwood chips as bedding in a barrier-sustained animal room at 24 ± 2°C and 55% humidity on a 12-hour light/dark cycle. The pitavastatin, (+)-monocalcium bis{(3*R*,5*S*,6*E*)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolyl]-3,5-dihydroxy-6-heptenoate} ($C_{50}H_{46}CaF_2N_2O_8$, MW 880.98), was kindly provided by Kowa Pharmaceutical Co., Ltd. Pitavastatin was well mixed at the concentrations of 20 and 40 ppm in AIN-76A powdered basal diet (CLEA).

Animal Experimental Schedule

To investigate the effects of pitavastatin on intestinal polyp formation, male Min mice at 6 weeks of age were given 0, 20, 40 ppm of pitavastatin in the diet for 14 weeks. Min mice were divided into groups of 20. With 20 ppm of pitavastatin, 4 mice died during the experiment. Food and water were available *ad libitum*. The animals were observed daily for health appearance and mortality. Body weights and food consumption were measured weekly. Animals were anesthetized with ether and sacrificed, and blood samples were collected from the caudal vena cava. Serum levels of TG and total cholesterol were measured as reported previously (6). The experiments were carried out according to the "Guidelines for Animal Experiments in the National Cancer Center" and were approved by the Institutional Ethics Review Committee for Animal Experimentation in the National Cancer Center.

The intestinal tract was removed and separated into the small intestine, cecum, and colon. The small intestine was divided into the proximal segment (4 cm in length), and then the proximal (middle) and distal halves of the remainder. Polyps in the proximal segments were counted and all polyps were picked up under a stereoscopic microscope and the remaining intestinal mucosa (nonpolyp part) was removed by scraping, and then both stored at −80°C for the further real-time PCR analysis. Other segments were opened longitudinally and fixed flat between sheets of filter paper in 10% buffered formalin. The numbers and sizes of polyps and their distributions in the intestine were assessed with a stereoscopic microscope (6). A part of liver, femoral muscle, visceral fat, and right kidney were kept in 10% buffered formalin, and residues of liver, femoral muscle, visceral fat, and left kidney were frozen by liquid nitrogen and then stored at −80°C.

Determination of Serum Adipocytokine Levels

Serum samples from 20-week-old male Min mice with or without pitavastatin were measured for serum concentrations of adiponectin (R&D Systems), leptin (B-Bridge International, inc.), Pai-1 (Innovative) by an enzyme immunoassay and IL-1β, IL-6, MCP-1, TNFα, VEGF were determined by using Procarta Cytokine Assay mouse (Affymetrix, inc.) according to the manufacturer's protocol.

Immunohistochemical Staining

The middle segments of the small intestines were fixed, embedded, and sectioned as Swiss rolls for further immunohistochemical examination with the avidin–biotin complex immunoperoxidase technique. Polyclonal goat anti-COX-2 and anti-MCP-1 antibody (Santa Cruz Biotechnology), polyclonal rabbit anti-Pai-1 antibody (Santa Cruz Biotechnology), polyclonal rabbit anti-PPARγ antibody (Cell Signaling), monoclonal mouse anti-iNOS antibody, and anti-nitrotyrosin antibody (Santa Cruz Biotechnology) were used at 100x dilution. Polyclonal rabbit anti-8-nitroguanosine antibody (Cosmo Bio Co., Ltd.) and polyclonal goat anti-IL-6 antibody (Santa Cruz Biotechnology) were used at 50x dilution. As the secondary antibody, biotinylated anti-goat, -rabbit, and -mouse IgG (Vector Laboratories) were employed at 200x dilution. Staining was done using avidin biotin reagents (Vectastain ABC reagents; Vector Laboratories), 3,3′-diaminobenzidine and hydrogen peroxide, and the sections were counterstained with hematoxylin to facilitate orientation. As a negative control, consecutive sections were immunostained without exposure to the primary antibody.

Real-time PCR Analysis

Polyps and nonpolyp parts from proximal segments of small intestine of Min mice were rapidly deep-frozen in

Published OnlineFirst January 13, 2011; DOI:10.1158/1940-6207.CAPR-10-0028

Table 1. Number of intestinal polyps/mouse in Min mice with or without pitavastatin treatment

Pitavastatin (ppm)	No. of mice	Small intestine			Colon	Total
		Proximal	Middle	Distal		
0	20	4.6 ± 1.2	19.2 ± 5.2	46.2 ± 7.2	0.6 ± 0.4	70.5 ± 13.3
20	16	5.4 ± 1.8	13.3 ± 2.2	40.4 ± 6.6	0.9 ± 0.3	60.1 ± 10.1
40	20	5.1 ± 1.5	13.4 ± 3.2	27.4 ± 4.1**	0.5 ± 0.3	46.4 ± 8.0*

Data are mean ± SE

*, ** Significantly different from the pitavastatin untreated group at $P < 0.05$, $P < 0.01$.

liquid nitrogen and stored at −80°C. Total RNA was isolated from tissues by using Isogen (Nippon Gene), treated with DNase (Invitrogen) and 3-μg aliquots in a final volume of 20 μL were used for synthesis of cDNA using an Omniscript RT Kit (Qiagen) and an oligo (dT) primer. Real-time PCR was carried out using a DNA Engine Opticon 2 (MJ Japan) with SYBR Green Real-time PCR Master Mix (Toyobo) according to the manufacturer's instructions. Primers for mouse *adiponectin* (5′primer-AGGATGCTACTGTTGCAAGCTCTC, 3′primer-CAGTCAGTTGGTATCATGGTAGAG), *COX-2* (5′primer- AGAAGGAAATGGCTGCAGAA, 3′primer-GCTCGGCTTCCAGTATTGAG), *iNOS* (5′primer-CCGGCAAACCCAAGGTCTACGTT, 3′primer-CACATCCCGAGCCATGCGCACATCT), *IL-6* (5′primer-ACAACCACGGCCTTCCCTACTT, 3′primer-CACGATTTCCCAGAGAACATGTG), *MCP-1* (5′primer-CCACTCACCTGCTGCTACTCAT, 3′primer- TGGTGATCCTCTTGTAGCTCTCC), *Pai-1* (5′primer-GACACCCTCAGCATGTTCATC, 3′primer- GACTGTACAAATCACGTTGGGA), and *GAPDH* (5′primer-TTGTCTCCTGCGACTTCA, 3′primer-CACCACCCTGTTGCTGTA) were employed (28–31). To assess the specificity of each primer set, amplicons generated from the PCR reaction were analyzed for melting curves and also by electrophoresis in 2% agarose gels. Standard curves for absolute quantification were obtained with plasmids containing the various amplicons. From each plasmid a 10-fold dilution series was measured in duplicate. Quantification and generation of standard curves was carried out using a DNA Engine Opticon 2 (MJ Japan).

PPARγ Activity in Intestinal Mucosa and Liver

Nuclear extracts containing PPARγ from nonpolyp parts of intestinal mucosa and liver (50 mg each) of mice with or without 40-ppm pitavastatin treatment were prepared using NE-PER Nuclear and Cytoplasmic Extraction Reagents (PIERCE Biotechnology). PPARγ activation by pitavastatin treatment was assayed using an ELISA-based transactivation TransAM PPARγ kit (Active Motif) following the manufacturer's protocol.

Statistical Analysis

All the results are expressed as mean ± standard errors (SE) values, with statistical analysis using Dunnett's test and PPARγ activity in intestinal nonpolyp parts and liver were performed with Student's *t*-test. Differences were considered to be statistically significant at $P < 0.05$.

Results

Suppression of intestinal polyp formation in Min mice by pitavastatin treatment

Treatment with pitavastatin at doses of 20 and 40 ppm for 14 weeks did not affect body weights or health appearance of Min mice throughout the experimental period. Average daily food intake did not differ among the groups, being 2.39 ± 0.37 (mean ± SE), 2.49 ± 0.39, and 2.47 ± 0.25 g per mouse per day for the 0, 20, and 40-ppm groups of Min mice, respectively. No changes were observed in the liver, heart, kidney, and thymus weights that might have been attributable to toxicity.

Table 1 summarizes data on the number and distribution of intestinal polyps in the untreated and pitavastatin-treated groups. Almost all polyps developed in the small intestine, with only a few in the colon as reported previously (6). The treatment with pitavastatin at a dose of 40 ppm significantly reduced the total number of polyps to 65.8% ($P < 0.05$) of the value in the untreated group. Strong suppression of intestinal polyp development was observed at the distal parts of small intestine, with 41% reduction ($P < 0.01$) at 40 ppm. The maximum number of polyps was observed in the range of size from 0.5 mm to 2.5 mm in diameter. Treatment with 40-ppm pitavastatin significantly reduced the numbers of polyps ranging from 1.5 mm to 2.0 mm ($P < 0.05$ vs. 0 ppm; Fig. 1A). Small-size polyps (<2.0 mm) were mainly distributed in distal parts of the small intestine (Fig. 1B). The group treated with 40-ppm pitavastatin significantly reduced the number of small-size polyps (21.6 ± 1.9/mouse, $P < 0.01$) in distal part compared with the untreated group (33.9 ± 2.4/mouse; Fig. 1B). The number of large-size polyps (≥2.0 mm) in distal parts of the untreated group was 11.9 ± 3.5/mouse, and the number in 40-ppm pitavastatin–treated group was decreased to 5.4 ± 1.6/mouse ($P < 0.05$; Fig. 1C).

Serum lipid and adipocytokine levels in Min mice with pitavastatin treatment

Consistent with our previous reports (5–7), Min mice fed with the basal diet at 20 weeks of age were in the

Published OnlineFirst January 13, 2011; DOI:10.1158/1940-6207.CAPR-10-0028

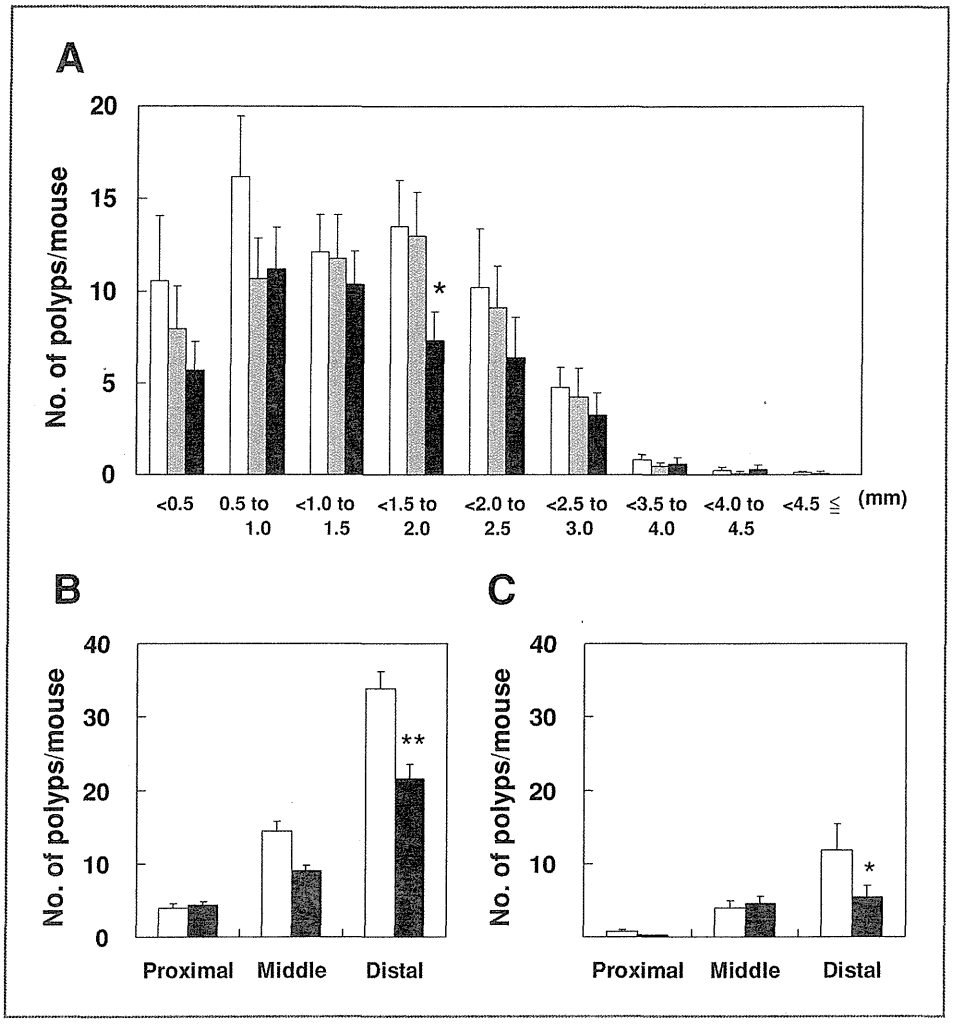

Figure 1. Effect of pitavastatin on the intestinal polyp size distribution in Min mice. Min mice were fed with a basal diet (open box) or a diet containing 20 ppm (gray box), 40-ppm (black box) pitavastatin. All of the intestinal polyps are grouped by size and its number/mouse is shown (A). Small intestinal polyps are sectioned into proximal, middle, and distal part, and the number/mouse having a diameter of less than 2 mm is shown in *B*, and 2 mm or more than 2 mm is shown in C. Date are mean ± SE, $^{*}P < 0.05$ vs. 0 ppm; $^{**}P < 0.01$ vs. 0 ppm.

hypertriglyceridemic state, with a TG level of 285.3 ± 64.3 mg/dL (mean ± SE). On the other hand, the TG level in wild-type mice was 32.7 ± 4.2 mg/dL. Treatment with 20 and 40-ppm pitavastatin slightly decreased serum levels of TG to 277.4 ± 68.1 mg/dL and 242.8 ± 49.9 mg/dL, respectively. However, these changes were not statistically significant. The level of total cholesterol had tendency to decrease to approximately 8%–9% of untreated group level and it did not seem to be influenced by dosage.

Serum concentrations of adiponectin, IL-1β, IL-6, leptin, MCP-1, TNFα, Pai-1, and VEGF were also measured to evaluate systemic effects of pitavastatin. Among the adipocytokines, IL-6, leptin, and MCP-1 were reduced significantly from 4.5 to 1.1 pg/mL, 2.5 to 1.4 ng/mL, and 12.1 to 7.7 pg/mL, respectively (Table 2).

COX-2, *iNOS* and adipocytokine mRNA levels in intestinal polyps and nonpolyp parts of Min mice treated with pitavastatin

To clarify the mechanisms of suppression on the development of intestinal polyps by pitavastatin treatment, mRNA expressions of *COX-2*, *iNOS*, and several adipocytokines in intestinal polyps and nonpolyp parts were investigated. Real-time PCR revealed that treatment with 20 and 40-ppm pitavastatin for 14 weeks effectively suppressed *COX-2*, *iNOS*, *IL-6*, *MCP-1*, and *Pai-1* mRNA levels in intestinal nonpolyp parts of Min mice (Fig. 2A and C). Treatment with 20-ppm pitavastatin reduced *COX-2*, *iNOS*, *IL-6*, *MCP-1*, and *Pai-1* mRNA levels to 15% ($P < 0.05$), 36% ($P < 0.05$), 36% ($P < 0.05$), 27%, and 73% of the untreated value, respectively, and 40 -ppm pitavastatin significantly reduced to 9% ($P < 0.01$), 22% ($P < 0.01$), 23% ($P < 0.01$), 15% ($P < 0.05$), and 18% ($P < 0.05$), respectively. Another adipocytokine, adiponectin, sustained basal mRNA level of wild-type in intestinal nonpolyp parts (data not shown). As shown in Figure 2B, mRNA expression levels of *COX-2* were higher in polyp than in nonpolyp parts. Treatment with 20 and 40-ppm pitavastatin slightly suppressed mRNA levels of *COX-2* in the polyps, but significantly suppressed its mRNA levels in nonpolyp parts.Meanwhile, treatment with 20 ppm of pitavastatin significantly suppressed *iNOS* mRNA levels in nonpolyp parts, and 40-ppm pitavastatin significantly suppressed both polyp and nonpolyp parts (Fig. 2C).

Published OnlineFirst January 13, 2011; DOI:10.1158/1940-6207.CAPR-10-0028

Table 2. Serum adipocytokine levels in Min mice with 40 ppm or without pitavastatin treatment

Pitavastatin (ppm)	Adiponectin (μg/mL)	IL-1β (pg/mL)	IL-6 (pg/mL)	Leptin (ng/mL)	MCP-1 (pg/mL)	Pai-1 (ng/mL)	TNFα (pg/mL)	VEGF (pg/mL)
0	12.20 ± 0.89	35.80 ± 8.00	4.51 ± 1.18	2.47 ± 1.80*	12.10 ± 1.30*	4.04 ± 2.41	5.90 ± 3.64	11.20 ± 1.51
40	13.90 ± 0.91	20.30 ± 6.20	1.11 ± 0.66*	1.38 ± 0.38*	7.66 ± 1.27*	3.32 ± 0.43	6.03 ± 2.62	8.90 ± 1.20

Data are mean ± SE ($n = 3\sim6$)

* Significantly different from the pitavastatin untreated group at $P < 0.05$.

Evaluation of nitrative stress in the intestinal polyp in Min mice treated with pitavastatin.

To evaluate the effects of iNOS overexpression as a nitrative stress in intestinal polyp in Min mice, localization of iNOS and the resultant nitration reaction were examined by immunohistochemistry using an anti-nitrotyrosin antibody and anti-8-nitroguanosine antibody. Nitrotyrosin was observed mainly in the stroma cells and 8-nitroguanosine was observed mainly in the cytoplasm of epithelial cells. Both nitrotyrosin and 8-nitroguanosine were weakly suppressed by 40-ppm pitavastatin treatment (Fig. 3A–F). In addition, localization and expression of COX-2 (Fig. 3G and H), IL-6, MCP-1, Pai-1, and PPARγ in the intestinal polyps were examined by immunohistochemistry. COX-2 was observed mainly in the stroma cells and IL-6, MCP-1, Pai-1, and PPARγ were observed mainly in the cytoplasm of epithelial cells without being affected by 40 ppm-pitavastatin treatment (Supplemental Fig. 1).

Effect of pitavastatin on PPARγ-DNA binding activity in intestinal nonpolyp parts and liver

Statins are reported to suppress some inflammatory adipocytokines through the PPARγ activation (32). Thus, we further evaluated the effect of pitavastatin on PPARγ activation in nonpolyp parts of the small intestine and liver of Min mice. Treatment with pitavastatin increased

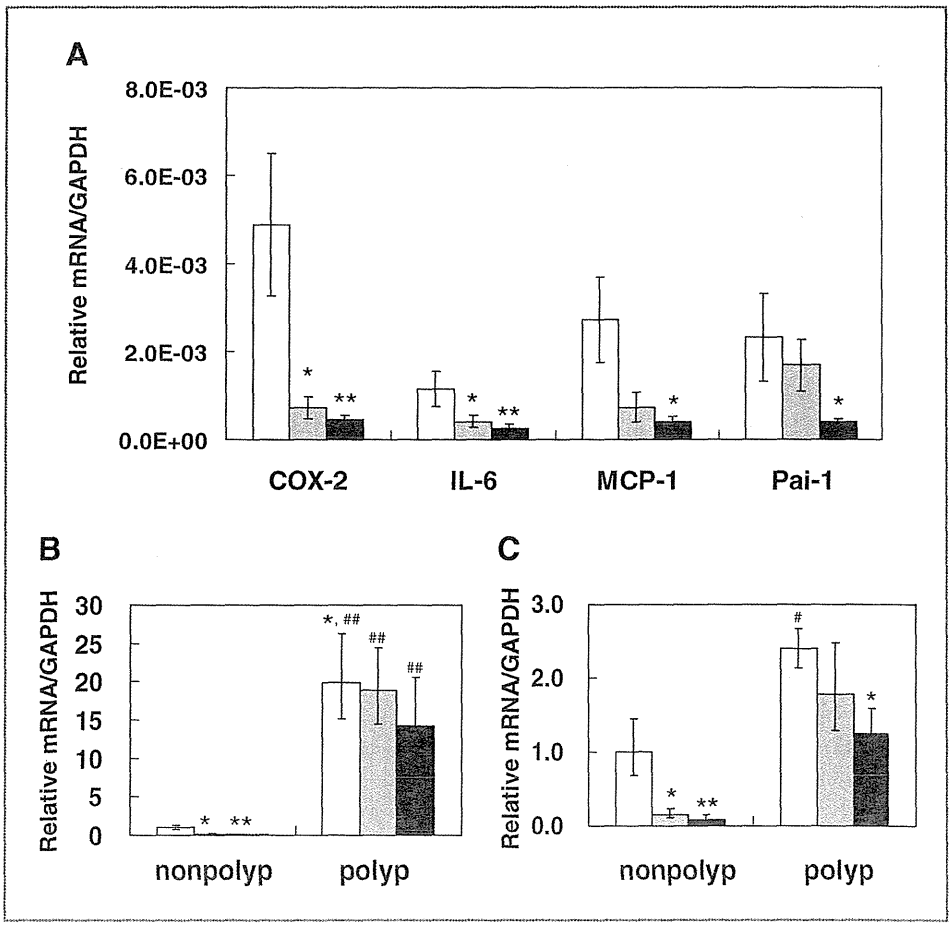

Figure 2. Changes of inflammation-related factors in intestinal nonpolyp parts and/or polyp parts of Min mice. Real-time PCR was conducted to detect *COX-2*, *IL-6*, *iNOS*, *MCP-1*, and *Pai-1* using intestinal tissue of Min mice with 0 (open box), 20 (gray box), and 40-(black box) ppm pitavastatin treatment. The data of molecule copy number are shown on the Y-axis (A). Regarding *COX-2* and *iNOS*, relative expression levels in the intestinal nonpolyp parts and polyp parts are shown in *B*, and *C*, respectively. Data are normalized with GAPDH. Data are mean ± SE, $n = 7$–11 (nonpolyp parts), $n = 5$ (polyps). *$P < 0.05$ vs. 0 ppm; **$P < 0.01$ vs. 0 ppm. #$P < 0.05$ vs. nonpolyp part (0 ppm); ##$P < 0.01$ vs. nonpolyp part (0 ppm).

Published OnlineFirst January 13, 2011; DOI:10.1158/1940-6207.CAPR-10-0028

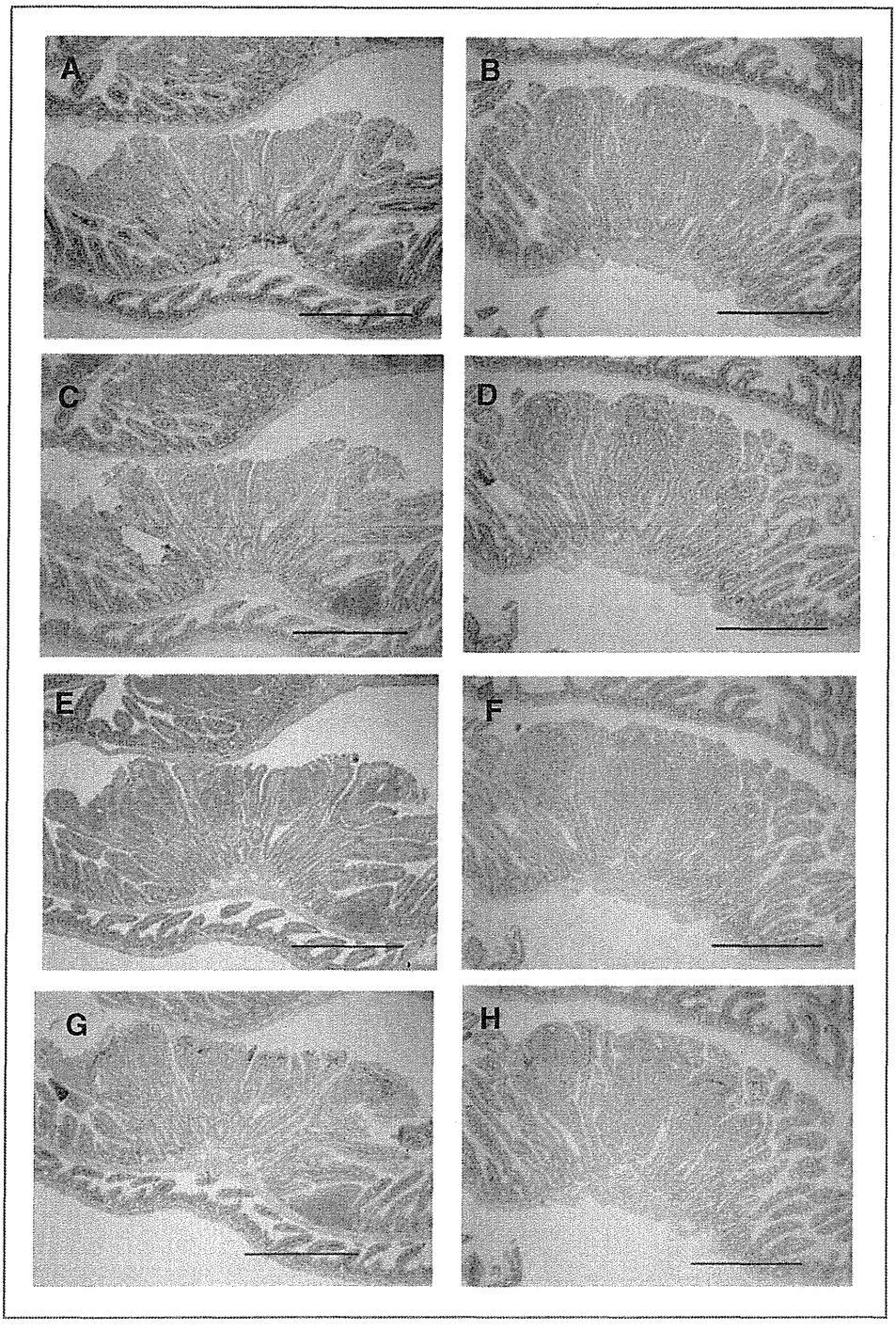

Figure 3. Modulation of mucosal oxidative/nitrosative stress by pitavastatin in Min mice. Immunohistochemical staining of iNOS (A and B), nitrotyrosine (C and D), 8-nitroganosine (E and F) and COX-2 (G and H), protein treated with (B, D, F and H) or without 40-ppm pitavastatin (A, C, E, and G), in Min mice. Bars represent 500 μm.

PPARγ-DNA binding activity in the intestinal nonpolyp parts and the liver at the dose of 40 ppm (Fig. 4A and B).

Discussion

In this study, it was showed that the treatment with pitavastatin suppressed intestinal polyp formation in Min mice with slight reduction of serum levels of total cholesterol and TG. The antiinflammatory effects were also observed in pitavastatin-treated Min mice, such as down regulation of *COX-2*, *iNOS*, and some adipocytokines including proinflammatory cytokines (*IL-6*, *MCP-1*, and *Pai-1*)mRNA levels. Moreover, guanosine nitration induced by reactive nitrogen oxides could be an important

Published OnlineFirst January 13, 2011; DOI:10.1158/1940-6207.CAPR-10-0028

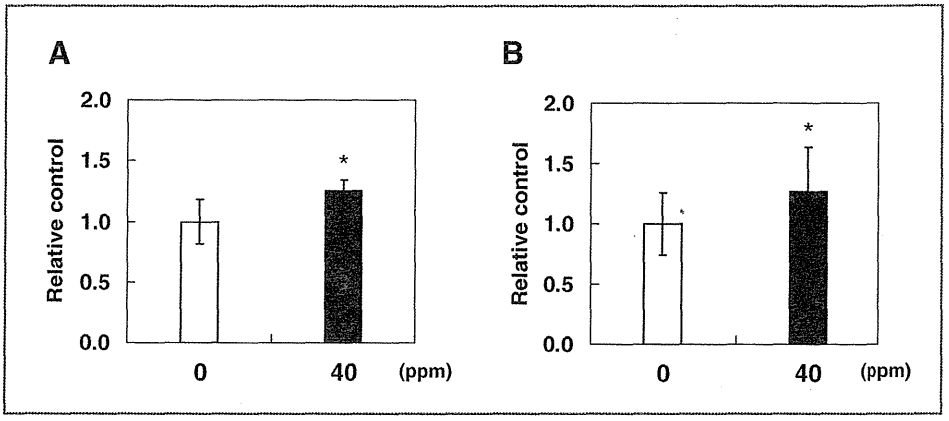

Figure 4. Changes of PPARγ-DNA binding activity by pitavastatin treatment in nonpolyp parts of the small intestinal and liver samples of Min mice. Nuclear fraction of small intestinal mucosa cells (A), and liver cells (B), were isolated and analyzed for PPARγ-DNA binding activity as described in Materials and Methods. Data are mean ± SE, $n = 9, 10$. *$P < 0.05$ vs. 0 ppm.

mediator of nitrative stress in the pathogenesis of intestinal polyp development in Min mice, and was reduced by pitavastatin treatment.

To date, lipid-lowering effects of statins have not been investigated in *Apc*-deficient mice model, which feature a hyperlipidemic state. Thus, we examined the effect of pitavastatin on serum lipid levels in Min mice and obtained a result of slight reduction. This result is similar to those shown in other rodent hyperlipidemic models. It has been shown that cholesterol synthesis enzymes were remarkably induced by feedback regulation in rodents (33), and a *de novo* cholesterol synthesis experiment by injection of ^{14}C-acetic acid showed that pitavastatin potently inhibits *de novo* cholesterol synthesis, without affecting serum lipids levels (34, 35). Taking these into consideration, HMG-CoA reductase activity might be inhibited by pitavastatin treatment in this study.

It has been reported that certain statins are able to exert antiinflammatory activities. Simvastatin inhibits proinflammatory gene expression by blocking nuclear factor kappa B (NFκB) signaling in intestinal epithelial cells, and attenuates dextran sodium sulfate-induced acute murine colitis (36). It has also been reported that pitavastatin inhibits NFκB activation and decreases IL-6 in human mammary carcinoma cells (37). Moreover, pitavastatin suppressed colitis-related colon carcinogenesis through modulation of mucosal inflammation with reduced nitrotyrosine-positivity (22). In this study, we have shown that clear downregulation of mRNA expression levels of *COX-2*, iNOS, and some adipocytokines (*IL-6*, *MCP-1*, and *Pai-1*) in the nonpolyp parts of the intestine by pitavastatin treatment, and significant reduction of *iNOS* mRNA level was observed in the polyp parts. These expression level changes of COX-2, iNOS, and adipocytokines, especially iNOS, could be associated with intestinal polyp development in Min mice. Indeed, it has been reported that iNOS inhibition, Pai-1 inhibition, COX-2 inhibition, and *IL-6* knock out suppressed intestinal polyp development in Min mice (31, 38, 39, 40). It has also been reported that 100-ppm atorvastatin treatment in Min mice slightly, but not significantly, reduced the activity and expression levels of COX-2 in the intestinal polyp (20). Expression of COX-2 was higher in polyp tissue than in nonpolyp parts, which may result in more resistance to pitavastatin's effects. INOS-dependent NO overproduction resulted in a nitration reaction, which takes place not only in tyrosine moieties of proteins but also in the nucleotide base guanosine, including RNA (41), and may account for the NO-induced cytotoxicity.

To further investigate the mechanisms of suppression of these proinflammatory genes by pitavastatin treatment, we focused on the levels of serum adipocytokines, including leptin, and activity of PPARγ, a member of the nuclear receptor superfamily. PPARγ, activated by statins (32), suppresses proinflammation gene expression (42). This study showed that pitavastatin treatment decreased serum leptin levels and increased PPARγ activity in the intestinal mucosa and the liver. It has been shown that simvastatin suppressed leptin expression in 3T3-L1 cells (43). Moreover, leptin induces iNOS and NO production (44), suggesting the interactions between leptin and NO. PPARγ activity induced by 40-ppm pitavastatin treatment might not be adequate to explain the reduction of adipocytokine levels by the same dose of pitavastatin treatment. As mentioned previously, NFκB signaling or other signaling may be additionally playing a role in the suppression of proinflammatory genes by pitavastatin treatment. In our previous study, a PPARγ ligand, pioglitazone, and an antiinflammatory drug, indomethacin, reduced intestinal polyps in Min mice (6, 45). Thus, it is assumed that PPAR activation and antiinflammatory activities of pitavastatin contribute, to some extent, to reduction of the development of intestinal polyps.

To explain the specific effect of pitavastatin on suppression of polyp development of the distal part in the small intestine, we investigated the expression levels of COX-2, IL-6, MCP-1, Pai-1, and PPARγ in the immunohistological study of distal and middle parts of the small intestine. However, the data did not show clear difference between the parts (data not shown). Further investigation is needed to clarify the differences between the distal and middle parts.

In conclusion, pitavastatin has potential benefit for suppression of intestinal polyp development. Thus,

Published OnlineFirst January 13, 2011; DOI:10.1158/1940-6207.CAPR-10-0028

pitavastatin might be a candidate for chemopreventive agent for human colon cancer.

Acknowledgments

This work was supported by Grants-in-Aid for Cancer Research, for the Third-Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health, Labour, and Welfare of Japan, and also from the Yakult Bio-science Foundation. S.T. is presently the recipient of a Research Resident Fellowship from the Foundation for Promotion of Cancer Research.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 10, 2010; revised November 19, 2010; accepted January 2, 2011; published OnlineFirst January 13, 2011.

Reference

1. Slattery ML, Boucher KM, Caan BJ, Potter JD, Ma KN. Eating patterns and risk of colon cancer. Am J Epidemiol 1998;148:4–16.
2. Matsuda T, Marugame T, Kamo K, Katanoda K, Ajiki W, Sobue T. The Japan Cancer Surveillance Research Group. Cancer incidence and incidence rates in Japan in 2002: based on data from 11 population-based cancer registries. Jpn J Clin Oncol 2008;38:641–8.
3. McKeown-Eyssen G. Epidemiology of colorectal cancer revisited: are serum triglycerides and/or plasma glucose associated with risk? Cancer Epidemiol Biomarkers Prev 1994;3:687–95.
4. Yamada K, Araki S, Tamura M, Sakai I, Takahashi Y, Kashihara H, et al. Relation of serum total cholesterol, serum triglycerides and fasting plasma glucose to colorectal carcinoma *in situ*. Int J Epidemiol 1998;27:794–8.
5. Niho N, Takahashi M, Kitamura T, Shoji Y, Itoh M, Noda T, et al. Concomitant suppression of hyperlipidemia and intestinal polyp formation in Apc-deficient mice by peroxisome proliferator-activated receptor ligands. Cancer Res 2003;63:6090–5.
6. Niho N, Takahashi M, Shoji Y, Takeuchi Y, Matsubara S, Sugimura T, et al. Dose-dependent suppression of hyperlipidemia and intestinal polyp formation in Min mice by pioglitazone, a PPAR gamma ligand. Cancer Sci 2003;94:960–4.
7. Niho N, Mutoh M, Takahashi M, Tsutsumi K, Sugimura T, Wakabayashi K. Concurrent suppression of hyperlipidemia and intestinal polyp formation by NO-1886, increasing lipoprotein lipase activity in Min mice. Proc Natl Acad Sci USA 2005;102:2970–4.
8. Endo A, Kuroda M, Tsujita Y. ML-236A, ML-236B, and ML-236C, new inhibitors of cholesterogenesis produced by Penicillium citrinium. J Antibiot (Tokyo) 1976;29:1346–8.
9. Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, et al. For the West of Scotland Coronary Prevention Study Group. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. N Engl J Med 1995;333:1301–7.
10. McTavish D, Sorkin EM. Pravastatin: a review of its pharmacology and use in the management of hypercholesterolaemia. Drugs 1991;42:65–89.
11. Todd PA, Goa KL. Simvastatin: a review of its pharmacology and use in the management of hypercholesterolaemia. Drugs 1990;40:583–607.
12. Plosker GL, Wagstaff AJ. Fluvastatin: a review of its pharmacology and use in the management of hypercholesterolaemia. Drugs 1996;51:433–59.
13. Lea AP, McTavish D. Atorvastatin: a review of its pharmacology and therapeutic potential in the management of hyperlipidaemias. Drugs 1997;53:828–47.
14. Olsson AG. Statin therapy and reductions in low-density lipoprotein cholesterol: initial clinical data on the potent new statin rosuvastatin. Am J Cardiol 2001;87:33B-6.
15. Poynter JN, Gruber SB, Higgins RD, et al. Statins and the risk of colorectal cancer. N Engl J Med 2005;352:2184–92.
16. ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group. The Antihypertensive and Lipid-Lowering Traetment to Prevnt Heart Attack Trial-Major outcomes in moderately hypercholesterolemic, hypertensive patients randomized to pravastatin vs usual care: the antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT-LLT). JAMA 2002;288:2998–3007.
17. Downs JR, Clearfield M, Weis S, Whitney E, Shapiro DR, Beere PA, et al. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS-Air Force/Texas Coronary Atherosclerosis prevention study. JAMA 1998;279:1615–22.
18. Narisawa T, Fukaura Y, Tanida N, Hasebe M, Ito M, Aizawa R. Chemopreventive efficacy of low dose of pravastatin, an HMG-CoA reductase inhibitor, on 1,2-dimethylhydrazine-induced colon carcinogenesis in ICR mice. Tohoku J Exp Med 1996;180:131–8.
19. Reddy BS, Wang CX, Kong AN, Khor TO, Zheng X, Steele VE, et al. Prevention of azoxymethane-induced colon cancer by combination of low doses of atorvastatin, aspirin, and celecoxib in F344 rats. Cancer Res 2006;66:4542–6.
20. Swamy MV, Patlolla JMR, Steele VE, Kopelovich L, Reddy BS, Rao CV. Chemoprevention of familial adenomatous polyposis by low dose of atorvastatin and celecoxib given individually and in combination to APC^{min} mice. Cancer Res 2006;66:7370–77.
21. Flores NA. Pitavastatin Nissan/Kowa Yakuhin/Novartis/Sankyo. Curr Opin Investig Drugs 2002;3:1334–41.
22. Yasui Y, Suzuki R, Miyamoto S, Tsukamoto T, Sugie S, Kohno H, et al. A lipophilic statin, pitavastatin, suppresses inflammation-associated mouse colon carcinogenesis. Int J Cancer 2007;121:2331–9.
23. Saito Y, Yamada N, Teramoto T, Itakura H, Hata Y, Nakaya N, et al. A randomized, double-blind trial comparing the efficacy and safety of pitavastatin versus pravastatin in patients with primary hypercholesterolemia. Atherosclerosis 2002;162:373–9.
24. Iglesias P, Diez JJ. New drugs for the treatment of hypercholesterolaemia. Expert Opin Investig Drugs 2003;12:1777–89.
25. Demierre MF, Higgins PDR, Gruber SB, Hawk E, Lippman SM. Statin and cancer prevention. Nat Rev Cancer 2005;5:930–42.
26. Wang CY, Liu PY, Liao JK. Pleiotropic effedts of statin therapy: molecular mechanisms and clinical results. Trends Mol Med 2008;14:37–44.
27. Moser AR, Pitot HC, Dove WF. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science 1990;247:322–4.
28. Inukai K, Nakashima Y, Watanabe M, Takata N, Sawa T, Kurihara S, et al. Regulation of adiponectin receptor gene expression in diabetic mice. Am J Endocrinol Metab 2005;288:E876–82.
29. Arita M, Yoshida M, Hong S, Tjonahen E, Glickman JN, Petasis NA, et al. Resolvin E1, an endogenous lipid mediator derived from omega-3 eicosapentanoic acid, protects against 2,4,6-trinitrobenzene sulfonic acid-induced colitis. Proc Natl Acad Sci USA 2005;102:7671–6.
30. Abe M, Matsuda M, Kobayashi H, Miyata Y, Nakayama Y, Komuro R, et al. Effects of statins on adipose tissue inflammation: their inhibitory effect on MyD88-independent IRF3/IFN-βpathway in macrophage. Arterioscler Thromb Vasc Biol 2008;28:871–7.
31. Mutoh M, Niho N, Komiya M, Takahashi M, Ohtsubo R, Nakatogawa K, et al. Plasminogen activator inhibitor-1 (Pai-1) blockers suppress intestinal polyp formation in Min mice. Carcinogenesis 2008;29:824–9.
32. Yano M, Matsumura T, Senokuchi T, Ishii N, Murata Y, Taketa K, et al. Statin activate peroxisome proliferators-activated receptor γ through extracellular signal-regulated kinase 1/2 and p38 mitogen-activated protein kinase-dependent cyclooxygenase-2 expression in macrophages. Circ Res 2008;100:1442–51.
33. Kita T, Brown MS, Goldstein JL. Feedback regulation of 3-hydroxy-3-methylglutaryl Coenzyme a reductase in livers of mice treated with mevinolin, a competitive inhibitor of the reductase. J Clin Invest 1980;66:1094–100.

Published OnlineFirst January 13, 2011; DOI:10.1158/1940-6207.CAPR-10-0028

34. Aoki T, Nishimura H, Nakagawa S, Kojima J, Suzuki H, Tamaki T,et al. Pharmacological profile of a novel synthetic inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. Arzneim-Forsch/Drug Res 1997;47:904–9.
35. Sata M, Nishimatsu H, Osuga J, Tanaka K, Ishizaka N, Ishibashi S, et al. Statins augment collateral growth in response to ischemia but they do not promote cancer and atherosclerosis. Hypertension 2004;43:1214–20.
36. Lee JY, Kim JS, Kim JM, Kim N, Jung HC, Song IS. Simvastatin inhibits NF-κB signaling in intestinal epithelial cells and amelio-rates2288;acute murine colitis. Int Immunopharmacol 2007;7:241–8.
37. Wang J, Kitajima I. Pitavastatin inactivate NF-κB and decreases IL-6 production through Rho kinase pathway in MCF-7. Oncol Rep 2007;17:1149–54.
38. Nakatsugi S, Fukutake M, Takahashi M, Fukuda K, Isoi T, Taniguchi Y, et al. Suppression of intestinal polyp development by nimesulide, a selective cyclooxygenase-2 inhibitor, in Min mice. Jpn J Cancer Res 1997;88:1117–20.
39. Baltgalvis KA, Berger FG, Pena MM, Davis JM, Muga SJ, Carson JA. Interleukin-6 and cachexia in $Apc^{Min/+}$ mice. Am J Physiol Regul Integr Comp Physiol 2008;294:R393–401.
40. Kohno H, Takahashi M, Yasui Y, Suzuki R, Miyamoto S, Kamanaka Y, et al. A specific inducible nitric oxide synthase inhibitor, ONO-1714 attenuates inflammation-related large bowel carcinogenesis in male $Apc^{Min/+}$ mice. Int J Cancer 2007;121:506–13.
41. Masuda M, Nishino H, Ohshima H. Formation of 8-nitroguanosine in cellular RNA as a biomarker of exposure to reactive nitrogen species. Chem Biol Interact 2002;139:187–97.
42. Leung WK, Bai AHC, Chan VYW, Yu J, Chan MWY, To K-F, et al. Effect of peroxisome proliferators activated receptor γ ligands on growth and gene expression profiles of gastric cancer cells. Gut 2004;53:331–8.
43. Maeda T, Horiuchi N. Simvastatin suppresses leptin expression in 3T3-L1 adipocytes via activation of the cyclic AMP-PKA pathway induced by inhibition of protein prenylation. J Biochem 2009;145:771–81.
44. Rodríguez A, Fortuño A, Gómez-Ambrosi J, Zalba G, Díez J, Frühbeck G. The inhibitory effect of leptin on angiotensin II-induced vasoconstriction in vascular smooth muscle cells is mediated via a nitric oxide-dependent mechanism. Endocrinology 2007;148:324–33.
45. Niho N, Mutoh M, Komiya M, Ohta T, Sugimura T, Wakabayashi K. Improvement of hyperlipidemia by indomethacin in Min mice. Int J Cancer 2007;121:1665–9.