adenocarcinomas [52] and also in 50% of BOP-induced pancreatic tumors in hamsters [53]. In addition, DCC expression is reduced or lost in poorly differentiated or undifferentiated pancreatic cancer cell lines, whereas it is conserved in the more differentiated ones [52,37].

p53 is the most frequently altered tumor suppressor gene in various cancers, its protein being a transcription factor which regulates cell cycle and apoptosis. *p53* is located at chromosome 17p and frequently inactivated by LOH and mutations in 40 to 75% of pancreatic adenocarcinomas in humans [34,45,54-56]. Overexpression of p53 protein can be detected in the nuclei of p53-mutated cells [54,55]. On the other hand, there is no evidence of p53 mutations in primary tumors in BOP-treated hamsters [57].

FHIT gene is a putative tumor suppressor gene located at chromosome 3p14, which is expressed in normal pancreatic ductular cells and is altered in pancreatic cancers [58]. Exogenous expression of *FHIT* in human pancreatic cancer cells causes cell cycle arrest and apoptosis [59] and loss of full length transcripts is frequent in primary pancreatic cancers of humans (62%) [58] and BOP-treated hamsters (73%) [60].

In addition to these gene alterations, increased protein expression, such as telomerase [61,62], midkine [63,64], cyclooxygenase-2 (COX-2) [65], metalloproteinase (MMP)-2, MMP-9 and membrane type 1-MMP [66,67] are shown in hamsters as in humans.

These findings indicate that multiple gene alterations and changes in protein expression observed in human pancreatic cancers are similarly involved in the BOP-induced hamster pancreatic ductal carcinogenesis model, underlining its utility for studying methods for pancreatic cancer prevention.

3. Modifying Factors in the Experimental Pancreatic Carcinogenesis Models

In addition to cigarette smoking, a well-known cause of pancreatic cancer, epidemiological studies have shown that chronic pancreatitis, obesity and diabetes mellitus are risk factors [68]. Using experimental animal models including mainly the BOP-induced pancreatic carcinogenesis model in hamsters, these and other possible promotive and suppressive factors in pancreatic carcinogenesis have been studied.

3.1. Obesity and Diabetes

Dietary fat has modifying effects on pancreatic carcinogenesis. It has been shown that a high-corn oil diet increased pancreatic ductal adenocarcinoma development in BOP-treated hamsters as compared with a low-corn oil diet [69]. Furthermore, a diet containing beef tallow has been shown to increase pancreatic cancer development compared with a diet containing corn oil [70]. Type and composition of fat are considered to be important. Fish oil rich in n-3 polyunsaturated fatty acids has been demonstrated to reduce pancreatic tumor incidences and hepatic metastasis in the BOP-treated hamster model [71]. Enhancing effects of high fat diet and suppressive influence of n-3 polyunsaturated fatty acid-rich fish oil on development of precancerous lesions, PanINs, in K-*ras* mutated GEM models have also been reported [72,73]. Obesity-mediated enhancement of PanIN lesion development is associated with increased inflammation, and abrogation of TNFR1 signaling blocks tumor promotion [72]. On the other hand, n-3 polyunsaturated fatty acids ameliorate

inflammation through inactivation of the NF-κB signaling pathway and inhibit cell proliferation through induction of cell cycle arrest and apoptosis [73,74].

Streptozotocin is known to induce diabetes through damage to islet cells and its modifying effects on pancreatic carcinogenesis have been studied in the BOP-treated hamster model, though the results are somewhat controversial. It has been reported that administration of streptozotocin alone caused islet cell tumors (44%), pseudoductules (40%), and ductular adenomas (12%), while simultaneous treatment with streptozotocin (single i.v. injection, 30 mg/kg body weight) and BOP (single s.c. injection, 10 mg/kg body weight) resulted in a significantly higher incidence of ductular carcinomas than induced by BOP alone [75]. On the other hand, pretreatment with streptozotocin at a diabetogenic dose (50 mg/kg body weight, three-times i.p. injection) prevented pancreatic cancer development when BOP was subsequently administered [76]. These inhibitory effects of pretreatment were dependent on the severity of the diabetes and could be blocked with nicotinamide [77]. These findings indicate that streptozotocin has a tumorigenic activity at relatively low dose, but when administered before BOP treatment, streptozotocin-induced diabetes/loss of insulin production could prevent BOP-induced pancreatic cancer development through killing islet cells. However, enhancing effects of diabetes and insulin-resistance observed in obesity on growth of transplantable pancreatic cancer cells are nevertheless convincing [78-80].

3.2. Pancreatitis

Cerulein is an analogue peptide of cholecystokinin, and its chronic intraperitorial injection causes pancreatic hypertrophy, characterized by increased pancreatic weight, increased amylase content and acinar cell hyperplasia. Moreover, cerulein augments the carcinogenicity of *N*-nitrobis(2-hydroxypropyl)amine (BHP) in the hamster pancreas [81]. It is also reported that chronic pancreatitis caused by cerulein induces development of pancreatic ductal adenocarcinomas in GEM mice expressing K-*ras*^{G12V} in acinar/centroacinar cells [31]. On the other hand, pancreatitis caused by common duct ligation before BOP injection decreased carcinoma development, while repeated induction of pancreatitis by common duct ligation after BOP administration resulted in enhanced development of carcinomas, with reference to both number and size [82].

Heavy alcohol drinking and cigarette smoking are major causes of pancreatitis in humans [83]. Epidemiological studies have shown that smoking and chronic pancreatitis are risk factors, whereas alcohol consumption itself has no direct relation [83,84]. However, in a transplacental induction model of pancreatic ductal cancer featuring NNK and EtOH treatment in the Syrian golden hamster, EtOH alone caused pancreatitis and hyperplasia, while NNK alone did not induce either [8], indicating a strong enhancing effect of pancreatitis on pancreatic carcinogenesis. It has also been reported that EtOH and nicotine promote pancreatic carcinogenesis in the DMBA-implanted mouse model [85,86].

In addition, repeated induction of pancreatitis with choline-deficient diet combined with DL-ethionine and L-methionine after initiation with BOP has been demonstrated to cause rapid production of pancreatic carcinomas in hamsters [87].

3.3. Others

There is limited evidence suggesting that red meat is a cause of pancreatic cancer [88,89]. In addition to total intake, the method of meat preparation is also important. Grilled red meat is a risk factor [90]. Effects of mutagenic heterocyclic amines (HCA) formed during cooking of meat on pancreatic carcinogenesis were studied in the BOP-treated hamster model. Among HCAs, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) and 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx) caused increase in pancreatic carcinoma development in BOP-treated hamsters [91]. Dietary intake of DiMeIQx has also been shown to be associated with pancreatic cancer risk in man [92].

High intake of fruits, vitamin C and vitamin E are suggested to protect against pancreatic cancer [88,93,94] and both vitamins have been found to exert protective effects on BOP-induced pancreatic cancer development in hamsters [95].

4. Cancer Prevention Targets for Humans and Evaluation in Experimental Pancreatic Carcinogenesis Models

From the etiology of pancreatic cancer, possible methods for prevention are: (1) avoiding carcinogenic *N*-nitrosoamine exposure such as cigarette smoke; (2) body weight control by diets and physical activity; (3) use of anti-hyperlipidemic and/or anti-diabetic agents; (4) use of anti-inflammatory agents.

Epidemiological studies have suggested that several agents having anti-hyperlipidemic, anti-diabetic or anti-inflammatory activities may have chemopreventive potential against pancreatic cancer [96]. Statins are cholesterol-lowering agents and also inhibit membrane-binding of the Ras protein, and are reported to reduce pancreatic cancer cell invasion and metastasis [97]. A case-control study of half a million veterans demonstrated a significant reduction of pancreatic cancer risk in statin users (adjusted OR = 0.37) [98], while meta-analysis of 12 studies showed no evidence of association between statin use and pancreatic cancer risk (RR = 0.88) [99]. Aspirin is a most frequently used nonsteroidal anti-inflammatory drug (NSAID) and has been reported to reduce cancer risk in several organs such as in the colon [100]. In the pancreas, epidemiological data on aspirin use are controversial [101,102]. A cohort study of post-menopausal women has shown that current use of aspirin is associated with a reduced risk of pancreatic cancer (adjusted RR = 0.57) [103], whereas another cohort study of nurses demonstrated that more than 20 years of regular aspirin use is associated with increased risk (RR = 1.58) [104]. Metformin, an anti-diabetic drug, activates AMP-activated protein kinase (AMPK) and inhibits pancreatic cancer growth [105,106]. A hospital-based case-control study has shown that metformin use is associated with reduced risk (OR = 0.38), while insulin or insulin secretagogue use is associated with increased risk (OR = 1.78) of pancreatic cancer in diabetic patients [107]. However, there is still no report of cohort study or randomized-control trial on metformin use. Since incidence of pancreatic cancer is relatively low compared with colon, breast and prostate cancers, prospective studies need quite a large population. In addition, randomized control studies are difficult, because the diseases such as hyperlipidemia and diabetes should be properly cared for. Therefore, evidences provided by preclinical studies including *in vivo* carcinogenesis studies using animal models are considered to be very important to evaluate the

chemopreventive efficacy and mechanisms of these agents. Factors related to insulin resistance and inflammation are candidate targets for pancreatic cancer prevention. Table 2 shows chemopreventive agents evaluated in BOP-induced pancreatic carcinogenesis.

Table 2. Chemopreventive agents of *N*-nitrosobis(2-oxopropyl)amine (BOP)-induced pancreatic carcinogenesis in hamsters.

Compounds	Mechanistic categories	Ref.
Anti-hyperlipidemic/diabetic agents		
Pioglitazone	PPARγ ligand	[113]
Metformin	AMPK activator	[114]
Anti-inflammatory agents		
Indomethacin	NSAID	[119]
Phenylbutazone	NSAID	[119]
NO-ASA	NO-NSAID	[121]
Nimesulide	COX-2 inhibitor	[118]
Celecoxib/Zileuton	COX-2/5-LOX inhibitors	[127]
ONO-1714	iNOS inhibitor	[131]
Others		
OPB-3206	MMP-2 inhibitor	[66]
Protochatechuic acid	Antioxidant	[135]
GTE	Antioxidant	[136]
BHA	Antioxidant	[137]
Sarcophytol A	Anti-tumor promoter	[138]
Methionine	Essential amino acid	[139]
PEITC	Cytochrome P450 suppressor	[140]
PPITC	Cytochrome P450 suppressor	[143]
PBITC	Cytochrome P450 suppressor	[144]
BITC	Cytochrome P450 suppressor	[145]
Sulforaphane	Anti-oxidative enzyme inducer	[145]
Aloe arborescens	Detoxyfiying enzyme inducer	[146]
Oltipraz	Nrf2 activator	[147]

4.1. Anti-Hyperlipidemic/Diabetic Agents

It has been reported that high cholesterol intake is associated with an increased risk of pancreatic cancer [108]. Smoking is associated with metabolic syndrome, and nicotine elevates serum triglyceride levels [109,110]. Obesity and diabetes are also closely associated with hyperlipidemia and hyperinsulinemia [111,112]. Interestingly, Syrian golden hamsters are in a hyperlipidemic state even under normal diet conditions, and pioglitazone, a ligand of peroxizome proliferator-activated receptor (PPAR) γ, has demonstrated to improve hyperlipidemia and suppress development of ductal adenocarcinomas in BOP-treated hamsters; the ductal adenocarcinoma incidences in the BOP + 800 ppm pioglitazone group and the BOP alone group were 38% *vs.* 80% ($P < 0.01$) and the multiplicities were 0.55 ± 0.15 *vs.* 1.37 ± 0.22 ($P < 0.01$), respectively [113]. In addition, the incidences of bile duct tumors in BOP-treated hamsters were clearly suppressed by pioglitazone [113]. Metformin, an activator of AMPK, has also been shown to decrease serum insulin levels and suppress development of

hyperplastic, dysplastic and malignant ductal lesions in the pancreas of BOP-treated hamsters on a high fat diet condition [114]. Pioglitazone and metformin are both anti-diabetic drugs which improve insulin resistance [115].

4.2. Anti-inflammatory Agents

Expression of COX-2 is up-regulated in PanIN and adenocarcinomas in humans and BOP-treated hamsters [64,116-118] and inhibition of prostanoid synthesis by NSAIDs, such as indomethacin and phenylbutazone, has been shown to reduce the development of precancerous lesions (atypical hyperplasia) and adenocarcinoma in the hamster model [119,120]. Whereas suppressive effects of aspirin were not significant, nitric oxide (NO)-donating aspirin, NO-ASA, has potent activity to prevent pancreatic cancer, especially arresting the transition from PanIN2 to PanIN3 and carcinoma, in BOP-treated hamsters [121]. It has also been reported that another COX-inhibitor, ibuprofen, reduces pancreatic cancer development in the hamster transplacental model with NNK + EtOH [122]. In GEM models, aspirin treatment has been shown to delay progression of PanINs in *LsL- Kras*^{G12D}*; Pdx1-Cre* mice and to partially inhibit development of invasive cancers in *LsL- Kras*^{G12D}*; LsL-Trp53*^{R172H}*; Pdx1-Cre* mice [123]. Furthermore, a selective COX-2 inhibitor, nimesulide, has been demonstrated to suppress development of precancerous lesions (atypical hyperplasia) and adenocarcinoma in BOP-treated hamsters [118]. In addition, inhibition of COX-2 by nimesulide delayed the appearance of PanIN-2 and PanIN-3 lesions in a conditional K*ras*^{G12D} mouse model, indicating the importance of prostaglandin synthesis by COX-2 in the early stage of pancreatic carcinogenesis [124]. In addition to COX-2, 5-LOX is also up-regulated in the ductal cells of PanIN and adenocarcinomas in humans, BOP-treated hamsters and Elastase-K*ras* mice [125,126]. Receptors of the downstream 5-LOX metabolite, leukotriene B_4, have been reported to be expressed in human pancreatic cancer tissues [125] and combination of COX-2-inhibition by Celebrex and 5-LOX-inhibition by Zyflo has shown to significantly decrease liver metastasis by pancreatic cancers in BOP-treated hamsters [127]. MK886, an inhibitor of 5-LOX activating protein FLAP, also reduced pancreatic cancer development in the hamster transplacental model with NNK + EtOH [122].

Increased expression of iNOS is also observed in pancreatic adenocarcinomas in humans and hamsters [128-131], perhaps involving K-*ras* activation [132]. Inhibition of iNOS by a selective iNOS inhibitor ONO-1714 suppressed development of precancerous lesions (atypical hyperplasia) and invasive adenocarcinomas in BOP-treated hamsters [131].

4.3. Others

Expression of MMP-2 is increased in precancerous lesions and adenocarcinomas, and proMMP-2 is highly activated in pancreatic carcinomas in humans and hamsters [133,66]. Inhibition of proMMP-2 activation by the MMP inhibitor OPB-3206 has been demonstrated to suppress pancreatic cancer development in BOP-treated hamsters under a rapid production protocol [66]. Another MMP inhibitor, RO 28-2653, has been reported to inhibit liver metastasis in the BOP-induced pancreatic carcinogenesis model, directly indicating roles for MMP-2 in cancer progression [134].

Protochatechuic acid, green tea extracts (GTE) and butylated hydroxyanisole (BHA) are anti-oxidative agents which have demonstrated inhibitory effects on pancreatic cancer development during

the post-initiation stage of the BOP-initiated hamster model [135-137]. Sarcophytol A, which is known to be an anti-tumor promoter, and methionine, which is an essential amino acid and associated with anti-oxidation, have also been shown to suppress pancreatic carcinogenesis in the BOP-treated hamster model [138,139].

Phenethyl isothiocyanate (PEITC), a natural constituent of cruciferous vegetables, has been demonstrated to be a potent chemopreventive agent in the initiation phase of pancreatic carcinogenesis in hamsters initiated with BOP [140,141], while not affecting the post-initiation phase [142]. Synthetic analogues of PEITC, such as 3-phenylpropyl isothiocyanate (PPITC), 4-phenylbutyl isothicyanate (PBITC) and benzyl isothicianate (BITC), and sulforaphane, Aloe arborescens and oltipraz have also been shown to suppress the initiation phase of BOP-induced pancreatic carcinogenesis through inhibition of activating (phase I) enzymes or activation of detoxifying (phase II) enzymes related to metabolism of BOP [143-147].

Nicotine-derived nitrosamine NNK stimulates release of noradrenaline/adrenaline by binding to alpha7 nicotinic acetylcholine receptors and activates beta-adrenergic receptors, resulting in proliferation of human pancreatic epithelial cells through cAMP-dependent signaling [148,149]. A beta-blocker propranolol has been shown to suppress the development of pancreatic cancer induced in the hamster transplacental model with NNK + EtOH [150].

Angiotensin-I-converting enzyme (ACE) and angiotensin II type 1 receptor are upregulated in human pancreatic cancer tissues and co-localized with vascular endothelial growth factor (VEGF) in malignant ducts and in stromal cells [151]. The ACE inhibitor enalapril has been demonstrated to delay progression of PanINs in *LsL- Kras^{G12D}; Pdx1-Cre* mice and to partially inhibit development of invasive cancer in *LsL- Kras^{G12D}; LsL-Trp53^{R172H}; Pdx1-Cre* mice [123].

An epidermal growth factor receptor inhibitor, gefitinib, has been demonstrated to suppress development of PanINs and cancer in *LsL- Kras^{G12D}; p48-Cre* mice [152]. Furthermore, a src kinase inhibitor, dasatinib, has been shown to suppress metastasis in *LsL- Kras^{G12D}; LsL-Trp53^{R172H}; Pdx1-Cre; Z/EGFP* mice, although there are no effects on proliferation and no survival advantage [153]. In addition, synthetic oleanane triterpenoids CDDO-methyl ester or CDDO-ethyl amide, the rexinoid LG100268, or the combination have been shown to increase survival in *LsL- Kras^{G12D}; LsL-Trp53^{R172H}; Pdx1-Cre* mice [154].

5. Conclusions

As shown above, the BOP-induced pancreatic carcinogenesis model in Syrian golden hamsters has genotypic and phenotypic similarities to the human case, and is a useful animal model for investigation of cancer prevention, even though the mechanistic analyses are a little difficult due to its limited genetic information. In this model, both precancerous lesions and advanced ductal carcimomas are assessable, and most of the BOP-treated hamsters develop pancreatic ductal carcinomas within six months. On the other hand, DMBA-induced pancreatic carcinogenesis models in rats and mice are considered to be not suitable for prevention studies, from the viewpoints of pathological origin of cancers and technical difficulty with neoplastic lesions developing only where carcinogen is implanted. GEM models are powerful for verifying the oncogenic mechanisms, but the process of carcinogenesis is pathologically different from the vast majority of human cases. Recently, several

chemoprevention studies using GEM models have been reported [73,123,124,126, 152-154], mainly of two types. One focuses on suppression of PanIN development in *LsL- Kras^{G12D}; Pdx1-Cre* mice or *LsL- Kras^{G12D}; p48-Cre* mice. In this system, incidences of pancreatic cancer are low (~20% at one year) [155], and therefore, it is difficult to obtain statistically significant results for cancer development. The PanIN lesions in GEM mice have similar phenotypes to humans, such as COX-2 [124] and LOX-5 [126] expression, but the pathological process of development of early lesions is quite different from human cases. Thus, the usefulness of this model may be limited regarding early detection and prevention of human pancreatic cancer. In suppression studies on cancer development or prolonged survival with *LsL- Kras^{G12D}; LsL-Trp53^{R172H}; Pdx1-Cre* mice, the GEM animals mimic the genetics of human pancreatic cancer and quickly develop pancreatic ductal carcinomas. This model may be more suitable for therapeutic studies than for prevention.

In humans, a number of epidemiological studies have suggested reduced pancreatic cancer risk with use of anti-hyperlipidemic/diabetic or anti-inflammatory agents. However, this is difficult to prove in randomized-control studies, because of the relatively low incidence of pancreatic cancer in humans and the absence of early biomarkers to predict pancreatic cancer. Thus, *in vivo* carcinogenesis studies using animal models are important to support the epidemiological findings and provide direct evidence. Some anti-hyperlipidemic and anti-inflammatory agents have indeed been shown to exert suppressive effects on pancreatic carcinogenesis in animal models including that with BOP-initiation in the hamster, indicating that factors related to hyperlipidemia, insulin resistance and inflammation are candidate targets for pancreatic cancer prevention.

Acknowledgements

The work performed in our laboratory was supported in part by: Grants-in-Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan and Management Expenses Grants from the Government to the National Cancer Center (21-2-1); a grant of the Research Grant of the Princess Takamatsu Cancer Research Fund (08-24009); a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (J.S.P.S.); and a grant of the Third-Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health, Labour and Welfare of Japan.

M. Hori is an Awardee of Research Resident Fellowship from the Foundation for Promotion of Cancer Research (Japan) for the Third-Term Comprehensive 10-Year Strategy for Cancer Control.

References

1. Matsuno, S.; Egawa, S.; Shibuya, K.; Shimamura, H.; Sunamura, M.; Takeda, K.; Katoh, H.; Okada, S.; Suda, K.; Nakao, A.; Isaji, S.; Hiraoka, T.; Hosotani, R.; Imaizumi, T. Pancreatic cancer: Current status of treatment and survival of 16071 patients diagnosed from 1981–1996, using the Japanese National Pancreatic Cancer Database. *Int. J. Clin. Oncol.* **2000**, *5*, 153-157.
2. Standop, J.; Schneider, M.B.; Ulrich, A.; Pour, P.M. Experimental animal models in pancreatic carcinogenesis: Lessons for human pancreatic cancer. *Dig. Dis.* **2001**, *19*, 24-31.
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.

GASTROENTEROLOGY 2011;140:2000-2008

BASIC—ALIMENTARY TRACT

Loss of Adiponectin Promotes Intestinal Carcinogenesis in *Min* and Wild-type Mice

MICHIHIRO MUTOH,* NAOYA TERAOKA,* SHINJI TAKASU,* MAMI TAKAHASHI,* KUNISHIGE ONUMA,* MASAFUMI YAMAMOTO,* NAOTO KUBOTA,‡ TAKAMOTO ISEKI,‡ TAKASHI KADOWAKI,‡ TAKASHI SUGIMURA,* and KEIJI WAKABAYASHI*,§

*Cancer Prevention Basic Research Project, National Cancer Center Research Institute, Tokyo, Japan; ‡Department of Diabetes and Metabolic Diseases, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; and §Graduate School of Food and Nutritional Sciences, University of Shizuoka, Shizuoka, Japan

BACKGROUND & AIMS: Metabolic syndrome- and obesity-associated cancers, including colon cancer, are common in Western countries. Visceral fat accumulation and decreased levels of plasma adiponectin (APN) have been associated with development of human colorectal adenoma. We investigated the function of APN in intestinal carcinogenesis. **METHODS:** $APN^{+/+}$, $APN^{+/-}$, or $APN^{-/-}$ mice (C57BL/6J) were given injections of azoxymethane (AOM), which led to development of intestinal tumors; these strains of mice were also crossed with *Min* mice to assess polyp formation. Adipocytokine levels and phosphorylation/activation of AMP-activated protein kinase (AMPK) were evaluated to investigate the mechanisms of APN in tumor growth. **RESULTS:** The total number of polyps in the intestines of male $APN^{+/-}$ *Min* and $APN^{-/-}$*Min* mice increased 2.4- and 3.2-fold, respectively, by the age of 9 weeks and 3.2- and 3.4-fold, respectively, by 12 weeks, compared with those of $APN^{+/+}$*Min* mice. Similar results were obtained from female mice. AOM induced colon tumor formation in 40% of $APN^{+/+}$, 50% of $APN^{+/-}$, and 71% of $APN^{-/-}$ ($P < .05$) mice, respectively; mean values for tumor multiplicity of each genotype were 0.5, 0.6, and 1.1 ($P < .05$), respectively. Phosphorylation of AMPK decreased in intestinal epithelial cells of $APN^{-/-}$ mice compared with $APN^{+/+}$ mice. Among serum adipocytokines, plasminogen activator inhibitor−1 levels increased in $APN^{-/-}$*Min* mice and $APN^{-/-}$ mice that received injections of AOM. Activation of AMPK suppressed expression of plasminogen activator inhibitor−1 in *Min* mice. **CONCLUSIONS: Mice with disruptions in *APN* develop more intestinal tumors and have decreased activation (phosphorylation) of AMPK and increased levels of plasminogen activator inhibitor−1, compared with wild-type mice. APN and its receptor might be developed as targets for cancer chemopreventive agents.**

Keywords: *Apc*-Deficient Mice; Adipokine; Colorectal Cancer; Chemoprevention

The criteria for metabolic syndrome include obesity, hyperlipidemia, type 2 diabetes, and hypertension. Several cancers, including colon cancer, are demonstrated to be associated with metabolic syndrome.[1-5] Obesity-associated cancers are common in Western countries, and they are currently increasing in Eastern countries as well. However, the mechanisms underlying how metabolic syndrome is associated with carcinogenesis remain to be fully understood. Insulin resistance, with hyperinsulinemia, hyperlipidemia, and hyperglycemia, are suggested to be involved in the promotion of colon cancer growth. In addition, dysregulation of adipocytokines, such as adiponectin (APN), leptin, plasminogen activator inhibitor-1 (PAI-1), and tumor necrosis factor−α (TNFα) has been shown to play a crucial role in the pathogenesis of the metabolic syndrome and postulated to promote carcinogenesis.[6] In human clinical studies, it has been reported that the amount of visceral fat positively correlates with colon adenoma risk, and serum APN levels show a negative correlation.[7]

APN is present at high levels in plasma (range, 3−30 μg/mL) as multimers. Both plasma APN and APN messenger RNA (mRNA) in adipose tissue are inversely correlated with body mass index and whole-body adipose mass. Furthermore, a decrease in plasma APN levels is associated with insulin resistance, type 2 diabetes, and coronary artery disease. Physiological functions of APN are elicited through 2 isoforms of its receptor, Adipo-R1 and Adipo-R2, stimulating AMP-activated protein kinase

Abbreviations used in this paper: ACF, aberrant crypt foci; AMPK, AMP-activated protein kinase; AOM, azoxymethane; APN, adiponectin; CK2β, casein kinase 2β; IL-1β, interleukin-1β; MCP-1, monocyte chemotactic protein-1; mRNA, messenger RNA; Pai-1, plasminogen activator inhibitor−1; PCR, polymerase chain reaction; RACK1, receptor for activated protein C kinase 1; TG, triglyceride; TNFα, tumor necrosis factor−α.

0016-5085/$36.00
doi:10.1053/j.gastro.2011.02.019

(AMPK) and peroxisome proliferator–activated receptor-α, respectively.[8]

Recently, we reported an age-dependent hypertriglyceridemic state with low expression levels of hepatic and intestinal lipoprotein lipase mRNA in *Apc*-deficient *Min* and *Apc*1309 mice, animal models of familial adenomatous polyposis.[9,10] Lipoprotein lipase catalyzes the hydrolysis of triglyceride (TG). Moreover, adipocytokines including plasminogen activator inhibitor–1 (Pai-1) were found to be remarkably overexpressed in the livers of *Min* mice as compared to wild-type mice.[11] In addition, hepatic APN mRNA levels were down-regulated in *Min* mice. Administration of Pai-1 blockers, SK-216 or SK-116, demonstrated the involvement of Pai-1 in the production of number of intestinal polyps.

It is assumed that adipocytokines have an impact on carcinogenesis. However, little is known about how their altered regulation is related to the development and progression of colon cancers. Thus, in the present study, we mated APN-deficient C57BL/6J mice with *Min* mice to investigate the effect of genetic inactivation of APN on intestinal carcinogenesis. APN deficiency resulted in increased intestinal polyp development. Moreover, a similar contribution was evident when APN-deficient C57BL/6J mice were treated with azoxymethane (AOM) to induce colon cancer. Reduced phosphorylated(p)-AMPK levels and increased p-Akt levels were suggested to be involved in the accelerated development of intestinal tumors. Moreover, the mechanistic consequences derived from the altered adipicytokines, APN and Pai-1, were demonstrated.

Materials and Methods

Animals

APN-deficient mice (C57BL/6J mice background) were generated as described previously and their genotypes were confirmed by polymerase chain reaction (PCR).[12] Both sexes were used at 6 weeks of age. Female C57BL/6-*Apc*$^{Min/+}$ mice (*Min* mice), 5 weeks of age, were purchased from The Jackson Laboratory (Bar Harbor, ME) and genotyped by the method reported previously. Heterozygotes of the female *Min* mice were mated with *APN*$^{-/-}$ C57BL/6J males to generate *APN*$^{+/-}$*Min* mice. Such males were crossed again with *APN*$^{+/-}$ C57BL/6J females to give *APN*$^{-/-}$*Min* mice. Offspring were genotyped by PCR.[9] In all the animal experiments in the present study, a maximum of 5 animals were housed per plastic cage, with sterilized softwood chips as bedding, in a barrier-sustained animal room, air-conditioned at 24 ± 2°C and 55% humidity, on a 12-hour light-to-dark cycle. AIN-76A powdered basal diet (CLEA Japan, Tokyo, Japan) and water were available ad libitum. The animals were observed daily for clinical signs and morbidity, and body weights and food consumption were measured weekly. At the sacrifice time point, mice were anesthetized with ether, and blood samples were collected from the abdominal vein. The experiments were conducted 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.

Experimental Protocol for APN-Deficient Min *and C57BL/6JMice*

Both sexes of *Min* mice (n = 7) with *APN*$^{+/+}$, *APN*$^{+/-}$, and *APN*$^{-/-}$ genotypes or C57BL/6J mice (n = 4) with *APN*$^{+/+}$, *APN*$^{+/-}$, and *APN*$^{-/-}$ genotypes were used for examination at the ages of 9 and 12 weeks. The levels of serum TG and total cholesterol were measured as reported previously.[10] The liver, kidneys, heart, and spleen were weighed and tissue samples from the liver and intestine were rapidly deep-frozen in liquid nitrogen and stored at −80°C.

The stomach and intestinal tract were removed, filled with 10% buffered formalin, and separated into the stomach, small intestine, cecum, and colon. The small intestine was divided into the proximal segment (4 cm in length), and proximal (middle) and distal halves of the remainder. All segments were opened longitudinally and fixed flat between filter paper in 10% buffered formalin. The numbers and sizes of polyps, and their distributions in the intestine were assessed with a stereoscopic microscope. Slices of the liver, kidneys, heart, and spleen were embedded in paraffin, sectioned, and stained with H&E.

Experimental Protocol for APN$^{+/-}$Min *Mice With APN Treatment*

Recombinant full-length murine APN was produced and purified as described previously[13,14] and dissolved in saline at a concentration of 300 μg/mL for use. *APN*$^{+/-}$*Min* mice of both sexes were divided into an APN-injected group (n = 10 each) and saline-injected control group (n = 10 each). Their body weight was measured and 1.5 mg/kg APN or the same volume of saline was intraperitoneally injected once a week from the age of 6 weeks to 12 weeks (6 times) following the method used in the previous report.[15] The numbers and sizes of polyps, and their distributions in the intestine were examined.

AOM-Induced Colon Tumor Development in APN-Deficient C57BL/6J Mice

Six-week-old male *APN*$^{+/+}$, *APN*$^{+/-}$, and *APN*$^{-/-}$ C57BL/6J mice (n = 30 each) received AOM at a dose of 10 mg/kg body weight intraperitoneally once a week for 6 weeks. Male *APN*$^{+/+}$ and *APN*$^{-/-}$ C57BL/6J mice (n = 10 each) without AOM treatment were used for evaluating sporadic colorectal cancer development. After laparotomy at 55 weeks of age, the entire intestines were resected and opened longitudinally and the contents