Title
Second hand smoke stimulates tumor angiogenesis and growth

Journal
Cancer Cell, 4(3)

ISSN
1535-6108

Authors
Zhu, B Q
Heeschen, C
Sievers, R E
et al.

Publication Date
2003-09-01

Peer reviewed

Second hand smoke stimulates tumor angiogenesis and growth

Bo-qing Zhu,[2] Christopher Heeschen,[3] Richard E. Sievers,[1] Joel S. Karliner,[2] William W. Parmley,[1] Stanton A. Glantz,[1] and John P. Cooke[4,*]

[1]Division of Cardiology, Department of Medicine
[2]Cardiology Research, VA Medical Center
University of California, San Francisco, California 94143
[3]Molecular Cardiology, University of Frankfurt, Frankfurt, Germany
[4]Division of Cardiovascular Medicine, Stanford University School of Medicine, Stanford, California 94305
*Correspondence: john.cooke@stanford.edu

Summary

Exposure to second hand smoke (SHS) is believed to cause lung cancer. Pathological angiogenesis is a requisite for tumor growth. Lewis lung cancer cells were injected subcutaneously into mice, which were then exposed to sidestream smoke (SHS) or clean room air and administered vehicle, cerivastatin, or mecamylamine. SHS significantly increased tumor size, weight, capillary density, VEGF and MCP-1 levels, and circulating endothelial progenitor cells (EPC). Cerivastatin (an inhibitor of HMG-coA reductase) or mecamylamine (an inhibitor of nicotinic acetylcholine receptors) suppressed the effect of SHS to increase tumor size and capillary density. Cerivastatin reduced MCP-1 levels, whereas mecamylamine reduced VEGF levels and EPC. These studies reveal that SHS promotes tumor angiogenesis and growth. These effects of SHS are associated with increases in plasma VEGF and MCP-1 levels, and EPC, mediated in part by isoprenylation and nicotinic acetylcholine receptors.

Introduction

Passive exposure to second hand smoke (SHS) is associated with atherosclerosis and cancer (US Environmental Protection Agency, 1992; Glantz and Parmley, 1992). Each of these tobacco-induced diseases requires neovascularization for growth of the lesion. Tumor angiogenesis is a multifactorial process that is generally required for tumor growth (Hanahan and Folkman, 1996). As the tumor expands, central ischemia induces expression of angiogenic growth factors, e.g., vascular endothelial growth factor (VEGF) or fibroblast growth factor (FGF). These factors stimulate endothelial cells in the existing vasculature to proliferate and migrate through the tissue to form new endothelialized channels. Circulating endothelial progenitor cells (EPCs) may also contribute to tumor vascularity (Rafii, 2000). Genetic inhibition of EPC recruitment inhibits tumor neovascularization (Lyden et al., 2001).

In addition to the FGF and VEGF systems, we have recently implicated another pathway in pathological angiogenesis (Cooke and Bitterman, 2003). Nicotinic acetylcholine receptors (nAChRs) are expressed in neuronal, endothelial, and vascular smooth muscle cells. Stimulation of endothelial nAChRs induces proliferation and reduces apoptosis of endothelial cells; increases endothelial tube formation in vitro; and augments pathological angiogenesis in murine models of inflammation, cancer, and atherosclerosis (Heeschen et al., 2001, 2002, 2003).

Another modulator of angiogenesis is the class of isoprenoids, which are essential for membrane attachment and biological activity of GTPases Ras and RhoA. In addition to reducing cholesterol levels, the inhibitors of 3-hydroxy-3methyl-glutaryl-coenzyme A reductase (HMG-coA reductase inhibitors or statins) inhibit synthesis of the isoprenoids geranyl and farnesyl pyrophosphate. By doing so, statins may interrupt angiogenic signaling (Vincent et al., 2001). Statins interfere with post-receptor signaling of VEGFR by reducing geranylation of rho kinase (Vincent et al., 2001; Weis et al., 2002; Gingras et al., 2000). Statins inhibit the secretion of monocyte chemoattractant protein-1 (MCP-1) by inhibiting farnesylation of Ras protein (Vincent et al., 2001; Romano et al., 2000). This action of statins may interfere with angiogenesis, as monocytes (or monocyte-like cells that may transform into EPCs) mediate proangiogenic effects and have been shown to release angiogenic factors (Rehman et al., 2003).

Because nicotine is a major component of SHS, we hypothe-

SIGNIFICANCE

We find that second hand smoke stimulates tumor growth, in part by accelerating tumor angiogenesis, in a murine model of Lewis lung cancer. The effects of second hand smoke are associated with increases in plasma levels of angiogenic cytokines and circulating endothelial progenitor cells. The angiogenic effects of second hand smoke can be blocked by inhibition of the nicotinergic acetylcholine receptor (nAChR). We have previously shown that an endothelial nAChR mediates endothelial cell proliferation and migration, as well as modulating angiogenesis in vivo. HMG-coA reductase inhibition also suppresses tumor angiogenesis and growth in this model. These pathophysiological insights may lead to new therapeutic avenues to reduce tumor growth and angiogenesis.

sized that SHS would accelerate tumor angiogenesis. We hypothesized that this effect may be mediated by an increase in humoral (i.e., VEGF, MCP-1) or cellular (i.e., EPC) components of tumor angiogenesis. We further hypothesized that these effects of SHS would be blocked by antagonists of the nAChR or by inhibition of isoprenoid synthesis. The following study was undertaken to test these hypotheses.

Results

Effect of second hand smoke on tumor growth

Five groups of eight or nine mice each were exposed to clean room air or second hand smoke (SHS) for 17 days after subcutaneous implantation of Lewis lung cancer cells. In addition, some animals were administered mecamylamine (an inhibitor of nicotinic cholinergic receptors) or cerivastatin (an HMG-coA reductase inhibitor) by osmotic minipumps.

SHS increased tumor size (1.31 $\pm$ 0.43 cm^3 versus 0.25 $\pm$ 0.05 cm^3, SHS versus control; $p < .0005$) and weight (0.93 $\pm$ 0.27 g versus 0.17 $\pm$ 0.08 g; SHS versus control; $p < .0005$) (Figure 1). In the absence of SHS, statins had no effect on tumor size ($p = .468$) or weight ($p = .399$). Cerivastatin and mecamylamine both substantially blocked the effects of SHS, each blocking about two thirds of the increase in tumor size and weight (Figure 1).

Effects of SHS on tumor angiogenesis

Tumor vascularity was determined from cryosections of tumor tissue derived from mice that had received a systemic infusion of space-filling fluorescent microspheres via the left ventricle.

These studies revealed that capillary density was increased about 2-fold in mice exposed to SHS ($p < .0005$) (Figure 2A). Cerivastatin had no effect on tumor angiogenesis in mice breathing clean room air, but blocked about two-thirds of the increment in angiogenesis associated with SHS ($p = .018$). Mecamylamine was an even more effective inhibitor of tumor angiogenesis, nearly abrogating the entire increase in capillary density induced by SHS ($p < .0005$).

SHS and mediators of tumor angiogenesis

Plasma VEGF and MCP-1 levels were determined by immunoassay. SHS increased plasma VEGF levels more than 2-fold (Figure 2B). Cerivastatin had no effect in mice breathing clean room air, but tended to reduce VEGF levels (by 25%) in mice exposed to SHS ($p = .145$). Mecamylamine blocked about two-thirds of the increase in VEGF induced by SHS ($p < .0005$).

SHS doubled serum MCP-1 levels ($p < .0005$) (Figure 2C). Although cerivastatin had no effect in mice breathing clean room air, it blocked three-quarters of the increase in MCP-1 levels induced by SHS ($p < .0005$). Mecamylamine had a smaller, marginally significant ($p = .050$) effect on MCP-1 levels.

Circulating endothelial progenitor cells were detected by flow cytometry and were defined by positive staining for CD34 and flk-1. The number of circulating endothelial progenitor cells tripled in the SHS mice ($p < .0005$; Figure 2D). Cerivastatin had no significant effect on circulating endothelial progenitor cells. Mecamylamine blocked about one-third of the increase in circulating endothelial progenitor cells associated with SHS ($p < .0005$).

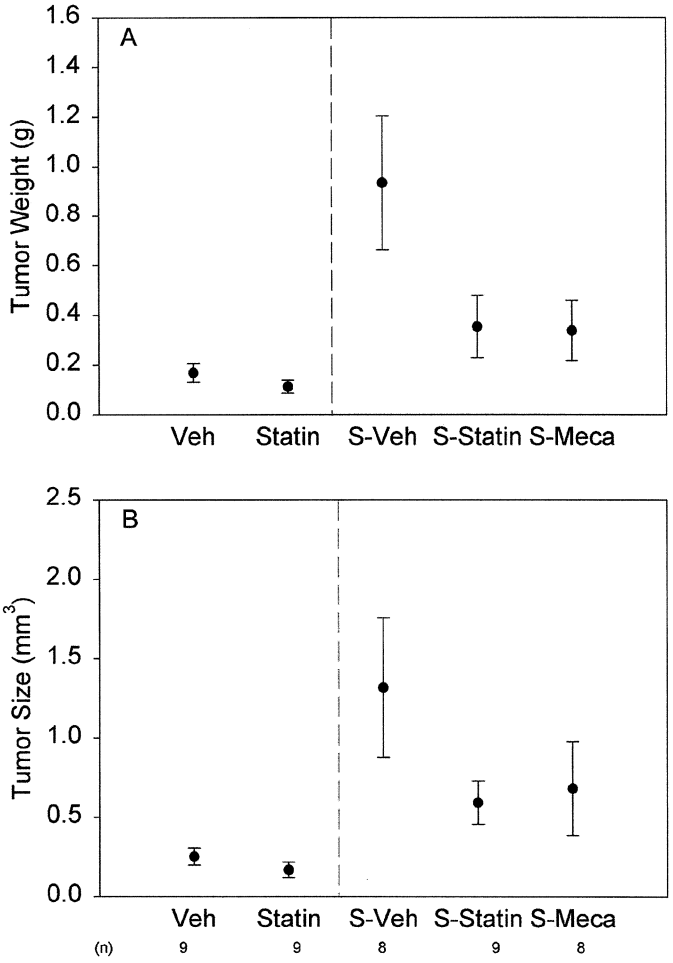

Figure 1. Second hand smoke (SHS) accelerates tumor growth

SHS substantially increased tumor size and weight in mice ($p < .0005$). Cerivastatin blocked about two-thirds of the effect of SHS ($p < .0005$ for SHS $\times$ Statin), as did mecamylamine ($p < .0005$). Statins did not have a significant effect in the absence of SHS ($p = .468$ for size and $p = .339$ for weight). Error bars indicate standard deviations.

Discussion

The salient findings of this study are: (1) second hand smoke (SHS) significantly increases tumor size, weight, and vascularity; (2) SHS increases serum VEGF and MCP-1 levels; (3) SHS increases the number of circulating endothelial progenitor cells; and (4) Antagonism of the nACh receptor, or inhibition of isoprenoid metabolism, reverses in part these effects of SHS. Specifically, the nicotine acetylcholine receptor antagonist, mecamylamine, reduced the effects of SHS on tumor size, weight, and vascularity, as well as levels of circulating MCP-1, VEGF, and endothelial progenitor cells. The HMG-CoA reductase inhibitor, cerivastatin, reduced the effects of SHS on tumor size, weight, and vascularity and reduced serum MCP-1 levels. Statins did not significantly modulate the effects of SHS on VEGF or circulating endothelial progenitor cells. Statins, at the dose we used, did not affect any of the variables we measured in mice breathing clean room air.

SHS, the tobacco combustion products inhaled by non-

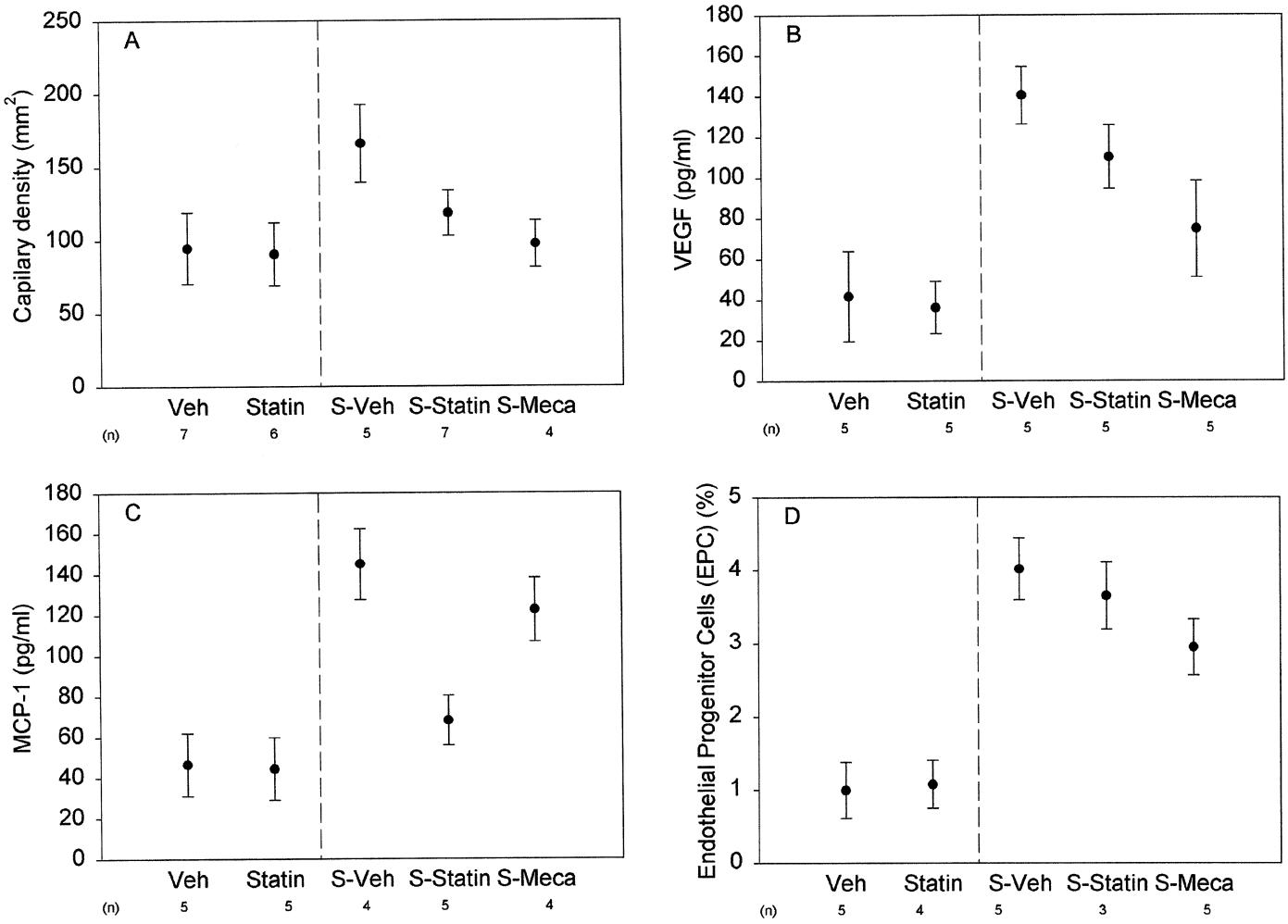

Figure 2. SHS promotes tumor angiogenesis

A: SHS increased capillary density in the tumors ($p < .0005$). Cerivastatin ($p = .018$ for SHS × statin) and mecamylamine ($p < .0005$) partially blocked this effect.

B: Plasma VEGF levels increased in mice exposed to SHS ($p < .0005$). Cerivastatin tended to inhibit this effect ($p = .145$ for SHS × statin). Mecamylamine blocked about two-thirds of the effect of SHS ($p < .0005$).

C: MCP-1 levels increased in mice exposed to SHS ($p < .0005$). Cerivastatin blocked most of this effect ($p < .0005$ for SHS × Statin); mecamylamine had a smaller effect ($p = .050$).

D: The number of circulating endothelial progenitor cells (EPS) increased with SHS ($p < .0005$). Cerivastatin did not significantly blunt this effect ($p = .271$ for SHS × Statin); mecamylamine blocked about one-third of the effect of SHS ($p < .0005$). Cerivastatin in the absence of SHS did not have a statistically significant effect on any variable ($p = .733$ for capillary density, $p = .637$ for VEGF, $p = .829$ for MCP-1, $p = .768$ for ECP). Error bars indicate standard deviations.

smokers in the proximity of burning tobacco, is dangerous because it contains high concentrations of nicotine, benzene, polycyclic aromatic hydrocarbons, fine particles ($PM_{2.5}$), and many other carcinogens and irritants (US Environmental Protection Agency, 1992; Glantz and Parmley, 1992; Cooke and Bitterman, 2003). The exposure chamber we used had an interior volume (3.6 m^3) similar to that of an automobile sedan (3.7 m^3). If four passengers each smoked four cigarettes per hour, that microenvironment would be similar to the one in the present study. The plasma cotinine concentrations we observed in our mice (150-200 ng/ml) are comparable to what would be expected from a heavy level of passive smoking, as in a casino, bingo parlor, or bar room. Our previous studies indicated that exposure to SHS at these levels increases experimental atherosclerosis in rabbits and myocardial infarct size in rats (Zhu et al., 1993, 1994).

We have observed anatomic and functional evidence that nicotine induces angiogenesis and accelerates the growth of tumor and atheroma in association with increased lesion vascularity, these effects mediated by nicotinic acetylcholine receptors (Heeschen et al., 2001, 2002, 2003). Signaling pathways mediating the effect of nicotine on endothelial network formation include phosphatidylinositol-3-kinase and mitogen-activated protein kinase pathways that converge on the activation of NF-κB (Heeschen et al., 2002). Our current results are consistent with the hypothesis that nicotine activates an endogenous angiogenic pathway mediated by endothelial nACh receptors. Furthermore, our observations indicate that nicotine absorbed from

SHS can exert a substantial effect on tumor angiogenesis and growth at levels of exposure experienced by people in smoky environments.

The effects of nicotine may be mediated in part by activation of endothelial-monocyte interactions involved in arteriogenesis. Nicotine has been shown to activate human monocyte-derived dendritic cells and to augment their capacity to stimulate T cell proliferation and cytokine secretion (Aicher et al., 2003). Furthermore, nicotine increases serum VEGF levels (Heeschen et al., 2001). These data are consistent with our observation that mecamylamine (an antagonist of nACh receptors) blocks the effect of SHS to increase serum VEGF and MCP-1 levels.

Mecamylamine (but not cerivastatin) blocked the effect of SHS to increase the number of circulating endothelial precursor cells. This may related to the effect of mecamylamine to block SHS-induced increases in serum VEGF levels, as administration of exogenous VEGF is known to stimulate the release of endothelial progenitors into the circulation (Rabbany et al., 2003). Alternatively, mecamylamine may block a direct effect of nicotine on mobilization of endothelial progenitors.

HMG-CoA reductase inhibitors (i.e., statins) such as cerivastatin and atorvastatin have a biphasic dose-dependent effect on angiogenesis that is independent of their effect on plasma cholesterol (Weis et al., 2002). At low doses, statins have a proangiogenic effect mediated by increases in NO synthase expression and activity (Weis et al., 2002). At high doses, statins strongly inhibit endothelial cell locomotion and capillary tube formation (Vincent et al., 2001; Weis et al., 2002), actions which may explain the antiangiogenic effects we observed. Furthermore, we find that cerivastatin blunts the increase in MCP-1 levels associated with SHS. This is consistent with the reports cited above (Vincent et al., 2001; Weis et al., 2002) and suggests that the anti-inflammatory effects of statins contribute to blocking the angiogenic effects of SHS. By inhibiting HMG-CoA reductase, statins block the formation of isoprenoid products of mevalonate. Isoprenoids such as geranylgeranyl pyrophosphate provide lipophilic anchors that are essential for membrane attachment and biological activity of small GTP binding proteins such as Rho A and Ras (Yoshida et al., 1991; Elson et al., 1999). Rho A is a small GTP binding protein crucial for the organization of the actin cytoskeleton and therefore for cell locomotion required for angiogenesis (Menager et al., 1999). Geranylgeranylation of Rho is required for its membrane localization and translocation and participation in angiogenic signaling (Park et al., 2002). Ras is involved in regulation of the mitogen-activated protein kinase and nuclear factor-kappa B pathways involved in angiogenesis (Finco et al., 1997; Berra et al., 2000; Shono et al., 1996). The antiangiogenic effects of the statins may explain the unanticipated observation that cancer deaths are reduced in some large trials of statins for prevention of cardiovascular events (Pedersen et al., 2000). However, with respect to the mechanisms by which SHS contributes to human malignancy, further work needs to be done to confirm the relevance of our observations. We used a murine cell line (Lewis lung cancer cells) to study tumor angiogenesis and growth in vivo. The cellular processes and factors mediating angiogenesis in naturally occurring human tumors may be different than those induced by tumor cell lines. For example, there is less evidence for an inflammatory response in human tumors than in those induced by transplanted cell lines in animals, indicating that spontaneous tumors are less immunogenic than experimental tumors (Hewitt et al., 1976).

In conclusion, exposure to SHS at levels observed in smoky environments stimulates tumor growth, tumor angiogenesis, and an increase in growth factors and cells known to contribute to tumor angiogenesis (Figure 3). These effects were reduced by mecamylamine, a nicotinic receptor antagonist, and cerivastatin, an inhibitor of isoprenoid synthesis. This study indicates that the oncogenic effects of SHS are mediated in part by angiogenic effects of nicotine.

Experimental procedures

Lewis lung cancer model

Lewis lung carcinoma cells (ATCC, Manassas, Virginia) were cultured in RPMI 1640 supplemented with 10% FBS. Lewis lung carcinoma cells (1×10^6 cells/mouse) were subcutaneously injected unilaterally into the right flank of C57BL/6J wild-type mice (8 weeks, 20–24 g).

Experimental groups

Forty three mice were randomized into five groups ($n = 8$ or 9 each group) after implantation of Lewis lung cancer cells: (1) clean room air-no drugs; (2) clean room air-statin (cerivastatin; Bayer, Germany); (3) SHS-no drugs; (4) SHS-statin; and (5) SHS-mecamylamine (a nicotinic acetylcholine receptor antagonist; Sigma, St. Louis, Missouri). The mice in the three SHS groups were exposed to sidestream tobacco smoke in an exposure chamber (model H 5500, BioClean, Duo Flo, Lab Product Inc.) that had a 3.6 m^3 interior volume, similar to that of an automobile sedan. Four Marlboro filter cigarettes were smoked using a smoking machine (RM 1/G, Heiner Borgwald GmbH, Hamburg, Germany) every 15 min, 6 hr/day, five days/week in the exposure chamber. The mice in the two non-SHS groups were exposed to clean room air. The mecamylamine (0.24 mg/kg per day) and cerivastatin (2.5 mg/kg per day) were administered by osmotic minipumps (Durect, Cupertino, California) subcutaneously implanted in the left lower dorsal area of the mice. After 17 days, all mice were sacrificed due to substantial tumor growth in animals of the vehicle-treated SHS group. The cotinine levels in the three SHS groups were similar and significantly higher than the two clean room air groups. There was no interaction of the drugs with cotinine levels.

Laboratory measurements

Tumor size was determined with a digital caliper ($4/3\ \pi \times$ length$/2 \times$ width$/2 \times$ thickness$/2$). Plasma cotinine levels, a measure of exposure to nicotine, were measured by STC Technologies (Bethlehem, Pennsylvania). Plasma VEGF levels were determined with a mouse VEGF ELISA kit (R&D Systems, Minneapolis, Minnesota). The MCP-1 levels were determined using a mouse JE/MCP-1 immunoassay (R&D Systems). Circulating endothelial progenitor cells were determined by flow cytometry and were defined by positive staining for CD34 (BD Pharmingen, San Diego, California) and flk-1 (BD Pharmingen). Data were analyzed using CellQuest software (Becton Dickinson), and all staining was referred to isotype-matched control antibodies purchased from BD Pharmingen. Tumor vascularity was determined in 10 μm cryosections of tumor tissue derived from mice that had received a systemic infusion of space-filling fluorescent microspheres (0.2 μm; Molecular Probes) via the left ventricle (Heeschen et al., 2001).

Statistical analysis

Values are presented as mean $\pm$ standard deviation. Statistical hypothesis testing was done with a general linear model:

$$y = b_{Control} + b_{SHS}\ SHS + b_{Statin}\ Statin + b_{SHSxStatin}\ SHSxStatin + b_{Meca}\ Meca$$

where y is the variable of interest and SHS = 1 if the mouse was exposed to secondhand smoke, Statin = 1 if the mouse received cerivastatin, and Meca = 1 if the mouse received mecamylamine; otherwise these variables were set to 0. $b_{Control}$ is the estimate of the dependent variable for animals breathing clean room air with no drugs, b_{SHS} is the change in y associated with SHS independent of the presence of cerivastatin or mecamylamine, b_{Statin} is the change associated with the statin, independent of the presence of SHS, $b_{SHSxStatin}$ is the SHS by Statin interaction (a measure of differential

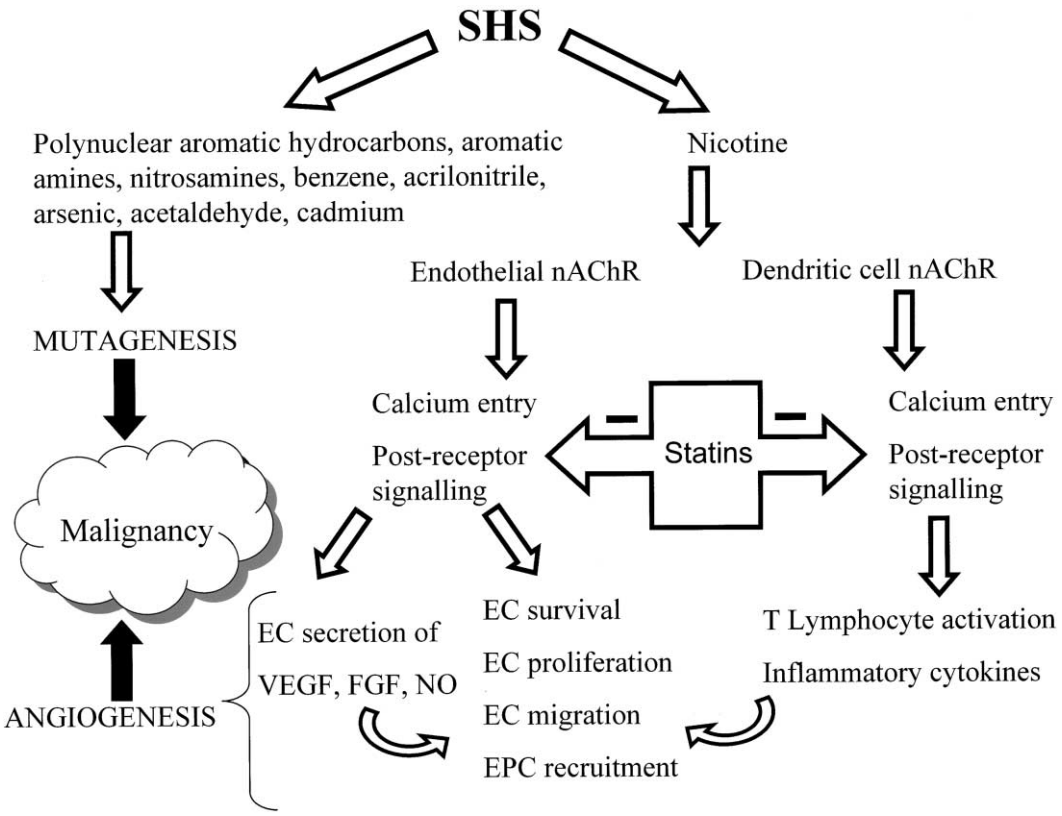

Figure 3. Mechanisms by which SHS promotes malignancy

SHS contains over 4000 compounds, some of which are mutagenic. In addition, nicotine in SHS stimulates nicotinic cholinergic receptors (nAChRs) on endothelial cells and monocytes to trigger calcium entry and signaling events. The post-receptor signaling is in part mediated by small GTPase proteins that require isoprenylation for their activity. The activity of these proteins (e.g., Ras and Rho) is inhibited by HMG-CoA reductase inhibitors, i.e., statins) that block their isoprenylation. Endothelial cells stimulated by nicotine release factors such as fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and nitric oxide (NO), which mediate angiogenesis. These agents, together with nicotine, enhance endothelial cell (EC) survival, proliferation, and migration and also recruit endothelial progenitor cells (EPCs). Stimulation of nAChRs on dendritic cells induces their activation of T lymphocytes and release of inflammatory mediators that contribute to EC activation. These processes favor angiogenesis, which promotes the vascularization and growth of malignant cells.

effects of the statin in the presence or absence of SHS), and b_{Meca} is the change associated with mecamylamine (in the presence of SHS since mecamylamine was only given to mice who were exposed to SHS). Raw observations are presented as mean ± standard deviation. p values are associated with each coefficient in the model.

Acknowledgments

This study was supported in part by grants from the National Heart, Lung, and Blood Institute (R01HL-586380), the Tobacco Related Disease Research Program (7RT-0128), Philip Morris Inc., the German Research Council (He 3044/1-1 and He 3044/2-2), the National Cancer Institute (CA-82103), and the Foundation for Cardiac Research, University of California, San Francisco. S.A.G. is a Cahan Distinguished Professor of the Flight Attendants Medical Research Institute, and J.P.C. is an established Investigator of the American Heart Association. Stanford University owns patents on the use of nicotinergic agonists or antagonists for therapeutic uses from which J.P.C. and C.H. derive royalties. This manuscript was partly presented at the American College of Cardiology 52nd Annual Scientific Session, March 30–April 2, 2003, Chicago, Illinois.

Received: July 8, 2003
Revised: August 4, 2003
Published: September 22, 2003

References

Aicher, A., Heeschen, C., Mohaupt, M., Cooke, J.P., Zeiher, A.M., and Dimmeler, S. (2003). Nicotine strongly activates dendritic cell-mediated adaptive immunity: potential role for progression of atherosclerotic lesions. Circulation *107*, 604–611.

Berra, E., Milanini, J., Richard, D.E., Le Gall, M., Vinals, F., Gothie, E., Roux, D., Pages, G., and Pouyssegur, J. (2000). Signaling angiogenesis via p42/p44 MAP kinase and hypoxia. Biochem. Pharmacol. *60*, 1171–1178.

Cooke, J.P., and Bitterman, H. (2003). Nicotine and angiogenesis: A new paradigm for tobacco-related diseases. Ann. Med., in press.

Elson, C.E., Peffley, D.M., Hentosh, P., and Mo, H. (1999). Isoprenoid-mediated inhibition of mevalonate synthesis: potential application to cancer. Proc. Soc. Exp. Biol. Med. *221*, 294–311.

Finco, T.S., Westwick, J.K., Norris, J.L., Beg, A.A., Der, C.J., and Baldwin, A.S., Jr. (1997). Oncogenic Ha-Ras-induced signaling activates NF-kappaB transcriptional activity, which is required for cellular transformation. J. Biol. Chem. *272*, 24113–24116.

Gingras, D., Lamy, S., and Beliveau, R. (2000). Tyrosine phosphorylation of

the vascular endothelial-growth-factor receptor-2 (VEGFR-2) is modulated by Rho proteins. Biochem. J. *348*, 273–280.

Glantz, S.A., and Parmley, W.W. (1992). Passive smoking causes heart disease and lung cancer. J. Clin. Epidemiol. *45*, 815–819.

Hanahan, D., and Folkman, J. (1996). Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell *86*, 353–364.

Heeschen, C., Jang, J.J., Weis, M., Pathak, A., Kaji, S., Hu, R.S., Tsao, P.S., Johnson, F.L., and Cooke, J.P. (2001). Nicotine stimulates angiogenesis and promotes tumor growth and atherosclerosis. Nat. Med. *7*, 833–839.

Heeschen, C., Weis, M., Aicher, A., Dimmeler, S., and Cooke, J.P. (2002). A novel angiogenic pathway mediated by non-neuronal nicotinic acetylcholine receptors. J. Clin. Invest. *110*, 527–536.

Heeschen, C., Weis, M., and Cooke, J.P. (2003). Nicotine promotes arteriogenesis. J. Am. Coll. Cardiol. *41*, 489–496.

Hewitt, H.B., Blake, E.R., and Walder, A.S. (1976). A critique of the evidence for active host defence against cancer, based on personal studies of 27 murine tumours of spontaneous origin. Br. J. Cancer *33*, 241–259.

Lyden, D., Hattori, K., Dias, S., Costa, C., Blaikie, P., Butros, L., Chadburn, A., Heissig, B., Marks, W., Witte, L., et al. (2001). Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. Nat. Med. *7*, 1194–1201.

Menager, C., Vassy, J., Doliger, C., Legrand, Y., and Karniguian, A. (1999). Subcellular localization of RhoA and ezrin at membrane ruffles of human endothelial cells: differential role of collagen and fibronectin. Exp. Cell Res. *249*, 221–223.

Park, H.J., Kong, D., Iruela-Arispe, L., Begley, U., Tang, D., and Galper, J.B. (2002). 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors interfere with angiogenesis by inhibiting the geranylgeranylation of RhoA. Circ. Res. *91*, 143–150.

Pedersen, T.R., Wilhelmsen, L., Faergeman, O., Strandberg, T.E., Thorgeirsson, G., Troedsson, L., Kristianson, J., Berg, K., Cook, T.J., Haghfelt, T., et al. (2000). Follow-up study of patients randomized in the Scandinavian simvastatin survival study (4S) of cholesterol lowering. Am. J. Cardiol. *86*, 257–262.

Rabbany, S.Y., Heissig, B., Hattori, K., and Rafii, S. (2003). Molecular pathways regulating mobilization of marrow-derived stem cells for tissue revascularization. Trends Mol. Med. *9*, 109–117.

Rafii, S. (2000). Circulating endothelial precursors: Mystery, reality, and promise. J. Clin. Invest. *105*, 17–19.

Rehman, J., Li, J., Orschell, C.M., and March, K.L. (2003). Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors. Circulation *107*, 1164–1169.

Romano, M., Diomede, L., Sironi, M., Massimiliano, L., Sottocorno, M., Polentarutti, N., Guglielmotti, A., Albani, D., Bruno, A., Fruscella, P., et al. (2000). Inhibition of monocyte chemotactic protein-1 synthesis by statins. Lab. Invest. *80*, 1095–1100.

Shono, T., Ono, M., Izumi, H., Jimi, S.I., Matsushima, K., Okamoto, T., Kohno, K., and Kuwano, M. (1996). Involvement of the transcription factor NF-kappaB in tubular morphogenesis of human microvascular endothelial cells by oxidative stress. Mol. Cell. Biol. *8*, 4231–4239.

US Environmental Protection Agency (1992). Respiratory health effects of passive smoking: Lung cancer and other disorders: US Environmental Protection Agency. Report No.: USEPA Document No. EPA/600/6–90/006F.

Vincent, L., Chen, W., Hong, L., Mirshahi, F., Mishal, Z., Mirshahi-Khorassani, T., Vannier, J.P., Soria, J., and Soria, C. (2001). Inhibition of endothelial cell migration by cerivastatin, an HMG-CoA reductase inhibitor: contribution to its anti-angiogenic effect. FEBS Lett. *495*, 159–166.

Weis, M., Heeschen, C., Glassford, A.J., and Cooke, J.P. (2002). Statins have biphasic effects on angiogenesis. Circulation *105*, 739–745.

Yoshida, Y., Kawata, M., Katayama, M., Horiuchi, H., Kita, Y., and Takai, Y. (1991). A geranylgeranyltransferase for rhoA p21 distinct from the farnesyltransferase for ras p21S. Biochem. Biophys. Res. Commun. *175*, 720–728.

Zhu, B.Q., Sun, Y.P., Sievers, R.E., Isenberg, W.M., Glantz, S.A., and Parmley, W.W. (1993). Passive smoking increases experimental atherosclerosis in cholesterol-fed rabbits. J. Am. Coll. Cardiol. *21*, 225–232.

Zhu, B.Q., Sun, Y.P., Sievers, R.E., Glantz, S.A., Parmley, W.W., and Wolfe, C.L. (1994). Exposure to environmental tobacco smoke increases myocardial infarct size in rats. Circulation *89*, 1282–1290.