SHORT COMMUNICATION

In Vivo Effects of Long-Term Cigarette Smoke Exposure on Mammary Tissue in Mice

Shannon Kispert, Susan Crawford, Grant Kolar, and Jane McHowat

From the Department of Pathology, Saint Louis University School of Medicine, St. Louis, Missouri

Accepted for publication
February 9, 2017.

Address correspondence to
Jane McHowat, Ph.D., Department
of Pathology, Saint Louis
University School of Medicine,
1402 S Grand, St. Louis,
MO 63104. E-mail:
mchowaj@slu.edu.

Cigarette smoking is the leading cause of preventable death worldwide and has been linked to the development and progression of cancer. Many cohort studies have described the link between patients with breast cancer and those with long-term smoking history. Despite the claim of correlation, the mechanism by which cigarette smoke alters normal breast epithelial cells and stroma and contributes to tumor cell growth remains undefined. To investigate whether cigarette smoke promotes ductal epithelial cell hyperplasia by stimulating stromal endothelial cell proliferation, we exposed mice to cigarette smoke for 6 months. We observed epithelial proliferation, increased fibrosis, increased vascularity, and mast cell infiltration. This is the first study to look at the *in vivo* changes in the breast after long-term cigarette smoke exposure and provides a novel insight to understanding how cigarette smoke contributes to early changes that may contribute to tumor formation and progression. In conclusion, this study suggests that cigarette smoke modulates key stromal-epithelial interactions to support increased angiogenesis, desmoplasia, and abnormal ductal epithelial cell growth. (*Am J Pathol* 2017, 187: 1238–1244; <http://dx.doi.org/10.1016/j.ajpath.2017.02.004>)

Cancer is a leading cause of death in the United States, contributing to 7.5 million deaths annually. Of all cancers, 90% are a result of environmental and lifestyle factors, such as poor diet, alcohol consumption, and cigarette smoking.^{1,2} Tobacco smoking is recognized as one of the most important modifiable risk factors worldwide.³ According to the Centers for Disease Control and Prevention, approximately 42 million (roughly 18%) of adults smoke cigarettes in the United States.¹ Smoking has been linked to several cancers, yet its association with breast cancer has remained uncertain. Several cohort studies have advocated for the role of cigarette smoking in breast cancer development and have shown that new cases of invasive breast cancer were higher in smokers than non-smokers.^{4–10} Despite recent attention, the issue remains controversial because of lack of consistent data and underlying mechanisms.

Breast cancer is the second most commonly diagnosed cancer behind nonmelanoma skin cancer, and serves as the second leading cause of cancer mortality among

women.¹¹ Initiation begins with a transforming event in a single cell, and the accumulation of changes combined with clonal expansion results in tumor formation.¹² Cells acquire sustained proliferative signaling, evade cell death, and subsequently enable replicative immortality.¹³ To keep the tumor alive, cells must induce angiogenesis to supply the tumor with blood and nutrients.¹⁴ The tumor cells are then able to invade the surrounding matrix, intravasate into circulation, and extravasate into distant sites to form micrometastasis. The first stage in defining breast cancer is typically ductal hyperproliferation with subsequent evolution *in situ*.¹⁵ However, usual hyperplasia (without atypia) has a low relative risk of approximately 1.8% for breast cancer, whereas atypical ductal hyperplasia has a much higher relative risk of 4%

Supported by the Saint Louis University Presidents Research Fund.
Disclosures: None declared.

and cumulative incidence of breast cancer of approximately 30% at 25 years follow-up.^{16–19}

There are approximately 7000 chemicals in cigarettes and at least 70 of them are carcinogenic, being capable of damaging cellular DNA and initiating tumorigenesis.¹ Components of cigarette smoke have been found in the DNA of epithelial cells of breast milk as well as nipple aspirate of female smokers, demonstrating that cigarette components access breast tissue.^{20,21} Our research group and others have shown increased motility and epithelial-mesenchymal transition in breast tumor cells exposed to components of cigarette smoke.^{22,23} Data to this point support plausible mechanisms by which cigarette smoke could lead to breast cancer development, yet a detailed mechanistic model remains undiscovered.

Herein, we have exposed mice to long-term cigarette smoking and determined its effect on mammary tissue. After 6 months of cigarette smoke exposure, the mammary tissue of mice exhibits increased ductal proliferation, increased vascularity, fibrosis, and mast cell infiltration. This novel pilot study provides, for the first time, a potential mechanism for changes that occur in response to cigarette smoke exposure to breast tissue by which primary breast tumor formation could occur.

Materials and Methods

Animal Cigarette Smoke Exposure

Six-month-old female C57/BL6 mice were used for all studies. Mice were exposed to cigarette smoke generated from the University of Kentucky 3R4F research cigarette with the SciReq InExpose system (Montreal, QC, Canada) using the Federal Trade Commission/International Standard Organization standard of 35-mL puffs of 2 seconds' duration taken once a minute. Mice were exposed to cigarette smoke for 48 minutes/day, 5 days/week for 24 weeks. Mice were sacrificed after 24 weeks, and mammary tissue was removed. All mice were age matched to room air controls (12 months of age at time of sacrifice). Data are representative of at least three mice for each group.

Immunohistochemistry

Mammary tissue for immunohistochemistry was removed from animals and fixed in 10% buffered formalin, embedded in paraffin, and cut into sections (5 μ m thick). Tissue was deparaffinized and rehydrated in xylene and decreasing concentrations of reagent alcohol. Sections underwent heat-induced antigen retrieval using a citric acid–based antigen unmasking solution (Vector Labs, Burlington, CA). Sections were incubated with blocking buffer and primary antibody [vascular endothelial growth factor (VEGF; 1:50;

Figure 1 Long-term cigarette smoke exposure causes epithelial growth and fibrosis in mouse mammary tissue. **A–H:** Mice were exposed to room air (**A, C, E, and G**) or 6 months of cigarette smoke (**B, D, F, and H**). Histological changes are seen in long-term smokers, which include epithelial growth (arrows), increased fibrosis (asterisks), and increased vascularity (arrowheads; **B** and **D**). Immunohistochemistry was performed for Ki-67 in mice after 6 months of cigarette smoke exposure (**F** and **H**) and room air controls (**E** and **G**). **I:** Mice exhibit more Ki-67–positive cells after 6 months of smoking than room air controls. Data are expressed as means $\pm$ SEM. * $P < 0.05$ versus room air control. Scale bars = 20 μ m (**A–H**). Original magnification, $\times 60$ (**A–H**).

Figure 2 Masson's trichrome stain for fibrosis and vimentin expression in mice exposed to cigarette smoke. **A–L:** Mice were exposed to room air (**A, B, E, F, I, and J**) or 6 months of cigarette smoke (**C, D, G, H, K, and L**) and Masson's trichrome was performed. **M–P:** Immunohistochemistry for vimentin expression in mice shows increased expression after 6 months of cigarette smoke exposure (**N and P**). **Q:** Image analysis of Masson's trichrome staining shows increased collagen deposition (blue in **A–P**) in mice after long-term cigarette smoke exposure. Data are means + SEM. * $P < 0.05$ compared with room air control. Scale bars: 200 μm (**A, C, E, G, I, and K**); 20 μm (**B, D, F, H, J, and L–P**). Original magnifications: $\times 10$ (**A, C, E, G, I, and K**); $\times 60$ (**B, D, F, H, J, and L–P**).

Abbiotec, San Diego, CA), pigment epithelium–derived factor (PEDF; 1:50; SantaCruz, Dallas, TX), vimentin (1:50; Cell Signaling Technology, Danvers, MA), anti-tryptase (1:50; Abcam, Cambridge, MA), Ki-67 (1:50; Cell Signaling Technology)] overnight.

Immunohistochemistry was completed with use of the Vectastain Elite Universal ABC system or horseradish peroxidase–conjugated secondary antibodies (Jackson Labs, Bar Harbor, ME) and 3,3'-diaminobenzidine (Bio-genex, Fremont, CA). Slides were counterstained with

filtered Gills III hematoxylin, blued in saturated lithium carbonate solution, and viewed under light microscope.

Statistical Analysis

All animal studies were repeated with at least three mice using at least three objective fields for analysis. Data were analyzed using *t*-test and are means + SEM. Differences were regarded as significant at $P < 0.05$.

Results

Long-Term Cigarette Smoke Exposure Promotes Epithelial Growth

Sustained proliferation of epithelial cells in the breast is important for tumor development. To determine the effect of cigarette smoke exposure on epithelial growth, we used mature female mice (6 months of age) and exposed them to the cigarette smoke or room air only for 6 months. Mice were sacrificed at 1 year of age, and we examined breast tissue from mice exposed to 6 months of cigarette smoke versus those exposed to room air only. We found that after 6 months of cigarette exposure, the mammary tissue exhibited increased ductal proliferation (Figure 1, B and D) when compared to room air animals (Figure 1, A and C). To investigate further, we performed immunohistochemistry for Ki-67, a cellular marker strictly associated with cellular proliferation (Figure 1, E–I). Tissue from animals exposed to cigarette smoke exhibited nearly a fourfold increase in Ki-67–positive cells when compared with room air controls (Figure 1I).

Matrix Deposition as a Result of Cigarette Smoke Exposure

Increased production as well as deposition of extracellular matrix proteins are risk factors in human breast cancer. After 6 months of exposure, mice exhibited periductal fibrosis as well as fat fibrosis, as seen via hematoxylin and eosin (Figure 1, B and D). To further investigate fibrosis, tissue from control and cigarette smoke–exposed animals was subjected to trichrome staining, to identify collagen deposition (Figure 2, A–L). We saw increased collagen staining surrounding the mammary ducts of animals with smoking history, which was approximately threefold that of room air controls (Figure 2Q). As another way to study matrix deposition, we examined vimentin expression on both animal groups. Immunohistochemical analysis showed increased vimentin expression in the breast after long-term cigarette smoke exposure (Figure 2, N and P) when compared to room air controls (Figure 2, M and O). These specialty stains suggest an increased matrix deposition in the breast after cigarette smoke exposure, which may contribute to tumor development.

Figure 3 Expression of vascular endothelial growth factor (VEGF) and pigment epithelium–derived factor (PEDF) after long-term cigarette smoke exposure in mice. **A–H:** Mice were exposed to room air (**A, C, E, and G**) or 6 months of cigarette smoke (**B, D, F, and H**). Immunohistochemistry shows increased VEGF expression in mice after 6 months of cigarette smoke exposure (**B and D**). Immunohistochemistry shows decreased PEDF expression in mice after 6 months of cigarette smoke exposure (**F and H**). **I:** Image analysis for PEDF and VEGF. Data are means + SEM. * $P < 0.05$ versus room air control. Scale bars: 200 μ m (**A, B, E, and F**); 50 μ m (**C, D, G, and H**). Original magnifications: $\times 10$ (**A, B, E, and F**); $\times 40$ (**C, D, G, and H**).

Increased Vascularity in Response to Cigarette Smoke

Increased vascularity is important in epithelial lesion progression and angiogenesis, both with great implications in breast pathology. We observed players involved

Figure 4 Mast cells in mice after long-term cigarette smoke exposure. **A–F:** Mice were exposed to room air (**A–C**) or 6 months of cigarette smoke (**D–F**). Hematoxylin and eosin and antitrypsin staining reveals increased numbers of mast cells (**arrows**) surrounding the ducts (**D**) and lymph nodes of mice (**E** and **F**) after 6 months of cigarette smoke exposure when compared to room air controls (**A–C**). **G:** Image analysis for tryptase immunohistochemistry. Data are means + SEM. Scale bars: 50 μ m (**C** and **F**); 20 μ m (**A**, **B**, **D**, and **E**). Original magnifications: $\times 40$ (**C** and **F**); $\times 60$ (**A**, **B**, **D**, and **E**).

in vascularity, including VEGF and PEDF. VEGF is the key mediator of angiogenesis in tumor formation and progression and thus is often found up-regulated in tumors. PEDF, a potent antiangiogenic factor, is often decreased or absent from developing tumors and its exogenous addition can inhibit tumor growth. After 6 months of cigarette smoke exposure, we saw a fourfold increase in VEGF expression (**Figure 3**, **B** and **D**) when compared to room air controls (**Figure 3**, **A** and **C**). This change in VEGF was accompanied by a PEDF expression of 12% mammary pad area to 2.5% mammary pad area (**Figure 3**, **F** and **H**) when compared to room air controls (**Figure 3**, **E** and **G**). These data were quantified by image analysis (**Figure 3I**).

Inflammation as a Result in Long-Term Cigarette Smoke Exposure

Chronic inflammation can be associated with tumor development and progression in many different neoplasms. Mast cells are critical players in the inflammatory process as well as angiogenesis. We observed an increase in mast cells after long-term cigarette smoke exposure surrounding ducts (**Figure 4D**) and lymph nodes (**Figure 4E**) when compared to room air controls (**Figure 4**, **A** and **B**). Tryptase, a marker for mast cell activation, was increased more than twofold in mouse mammary tissue after 6 months of cigarette smoke

exposure (**Figure 4**, **F** and **G**) when compared to room air controls (**Figure 4C**).

Discussion

Stromal-epithelial interactions are crucial in the development and progression of breast cancer. Studies have shown that genetic alterations in stromal cells may precede genotypic changes in epithelial cells.²⁴ We have seen stromal changes, which include an increase of mammary density, particularly collagen in the breast tissue (**Figure 2Q**). It is established that women with dense breasts have a significantly increased susceptibility to breast cancer development, and tumors most commonly arise in the denser portions of tissue containing collagen.^{25,26} *In vivo* studies have shown that collagen-dense breast in mice exhibit a threefold increase in tumor formation driven by the MMTV-PyVT transgene.²⁷ The increase in collagen observed after cigarette smoke exposure to mice may provide a mechanism for tumor development in the breast.

In addition to increased collagen, these areas of elevated breast density exhibit enhanced epithelial cellularity. Increased cell division has been well defined in tumorigenesis, including breast cancer. Increased proliferation of epithelial cells allows increased DNA damage to be propagated extensively.²⁸ We have found that exposure to

cigarette smoke leads to increased epithelial cellularity as well as high expression of Ki-67, a cellular marker for proliferation (Figure 1). Despite this well-known association between increased proliferation and neoplasia, the sources of mitogenic signals within normal tissue remain undefined.¹³

As mentioned at the beginning of the *Discussion*, the stromal-epithelial interactions are important in the development of tumors specifically in the breast. Mast cells are important to tissue injury and tend to accumulate at sites of tumor growth, specifically in the stroma.¹³ Mast cells could contribute to tumor growth through angiogenesis. Mast cells secrete IL-8, an angiogenic factor, as well as produce and secrete VEGF, a potent inducer of angiogenesis.^{29,30} We have found that breast tissue exposed to long-term cigarette smoke shows increased numbers of mast cells verified by detection of tryptase (Figure 4, D–F). This increase in mast cells could contribute to inflammation in the mammary tissue as well as induce angiogenesis.

Immune cells can synthesize and secrete angiogenic factors to promote new vessel formation during pathological conditions.³¹ Vessel formation can, in itself, support the migration of inflammatory cells throughout the breast. Mast cells can produce VEGF, but can also indirectly release VEGF from its fixed position in the extracellular matrix.²⁹ We observed increased vessel density as well as increased VEGF expression after long-term cigarette smoke exposure in the breast tissue of mice (Figures 1B and 3I). In addition to VEGF increase, we saw decreased expression of PEDF, a potent antiangiogenic factor (Figure 3I). PEDF mediates many biological pathways, including VEGF-driven angiogenesis, wherein PEDF inhibits VEGF binding with its receptor.³² Of note, the expression of VEGF positively correlates with the progression of neovascular diseases wherein PEDF levels diminish.³³

Biochemical pathways in the breast affected by cigarette smoke are unknown, yet several studies suggest possible mechanisms. Matrix metalloproteinases (MMPs) can degrade extracellular matrix components, promote cellular proliferation, and, of particular importance, act on PEDF by proteolysis, rendering its antiangiogenic properties null. As aforementioned, PEDF is a potent antiangiogenic factor and its depletion corresponds with VEGF up-regulation, and is often seen with tumor progression. MMP-2 can be induced by cigarette smoke via several mechanisms, including Janus kinase/STAT pathway and early growth response 1 induction.^{34,35} MMP-2 expression and activation can also be mediated by platelet-activating factor.^{36,37} We have shown previously that cigarette smoke exposure leads to platelet-activating factor accumulation in breast tumor cells and induces cell motility.²³ This platelet-activating factor accumulation could lead to MMP activation and PEDF down-regulation. Cigarette smoke–induced up-regulation of MMPs is just one plausible mechanism by which cigarette smoke could alter biochemical pathways that result in histological differences in the breast. Future studies will

focus on delineation of potential pathways and subsequent interventions to abrogate histological changes.

Tumor development from cigarette smoke exposure has been linked in several cancers, including lung, stomach, bladder, and esophagus. Several cohort studies describe the link between cigarette smoking and breast tumor development, yet no exact mechanism has been described. Our research group and others have shown that components of cigarette smoke can contribute to cell motility as well as induction of epithelial to mesenchymal transition in breast cancer cells. Despite these studies *in vitro*, there are no studies to date that look at the effect of long-term smoking on the breast *in vivo* from baseline. We have shown, for the first time, what effects long-term smoking has on mammary tissue *in vivo*. Our novel studies show that long-term exposure will result in increased epithelial growth, fibrosis, vascularity, and mast cell presence. These initial changes may play an important role in the development of a solid tumor.

References

1. HHS: The Health Consequences of Smoking—50 Years of Progress: A Report of the Surgeon General. Rockville, MD: US Department of Health and Human Services, Public Health Service, Office of the Surgeon General, 2014, p i
2. Anand P, Kunnumakkara AB, Kunnumakara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB: Cancer is a preventable disease that requires major lifestyle changes. *Pharm Res* 2008, 25:2097–2116
3. Agaku IT, King BA, Dube SR: Centers for Disease Control and Prevention (CDC): Current cigarette smoking among adults: United States, 2005–2012. *MMWR Morb Mortal Wkly Rep* 2014, 63:29–34
4. Reynolds P, Hurley S, Goldberg DE, Anton-Culver H, Bernstein L, Deapen D, Horn-Ross PL, Peel D, Pinder R, Ross RK, West D, Wright WE, Ziogas A: Active smoking, household passive smoking, and breast cancer: evidence from the California Teachers Study. *J Natl Cancer Inst* 2004, 96:29–37
5. Cui Y, Miller AB, Rohan TE: Cigarette smoking and breast cancer risk: update of a prospective cohort study. *Breast Cancer Res Treat* 2006, 100:293–299
6. Xue F, Willett WC, Rosner BA, Hankinson SE, Michels KB: Cigarette smoking and the incidence of breast cancer. *Arch Intern Med* 2011, 171:125–133
7. Gaudet MM, Gapstur SM, Sun J, Diver WR, Hannan LM, Thun MJ: Active smoking and breast cancer risk: original cohort data and meta-analysis. *J Natl Cancer Inst* 2013, 105:515–525
8. Olson JE, Vachon CM, Vierkant RA, Sweeney C, Limburg PJ, Cerhan JR, Sellers TA: Prepregnancy exposure to cigarette smoking and subsequent risk of postmenopausal breast cancer. *Mayo Clin Proc* 2005, 80:1423–1428
9. Al-Delaimy WK, Cho E, Chen WY, Colditz G, Willett WC: A prospective study of smoking and risk of breast cancer in young adult women. *Cancer Epidemiol Biomarkers Prev* 2004, 13:398–404
10. Luo J, Margolis KL, Wactawski-Wende J, Horn K, Messina C, Stefanick ML, Tindle HA, Tong E, Rohan TE: Association of active and passive smoking with risk of breast cancer among postmenopausal women: a prospective cohort study. *BMJ* 2011, 342:d1016
11. Siegel R, Naishadham D, Jemal A: Cancer statistics, 2013. *CA Cancer J Clin* 2013, 63:11–30
12. Polyak K: Breast cancer: origins and evolution. *J Clin Invest* 2007, 117:3155–3163

13. Hanahan D, Weinberg RA: Hallmarks of cancer: the next generation. *Cell* 2011, 144:646–674
14. Chaffer CL, Weinberg RA: A perspective on cancer cell metastasis. *Science* 2011, 331:1559–1564
15. Cai S, Sheng Z: Dedifferentiation: a new approach in stem cell research. *Bioscience* 2007, 58:655–662
16. Hartmann LC, Sellers TA, Frost MH, Lingle WL, Degnim AC, Ghosh K, Vierkant RA, Maloney SD, Pankratz VS, Hillman DW, Suman VJ, Johnson J, Blake C, Tlsty T, Vachon CM, Melton LJ, Visscher DW: Benign breast disease and the risk of breast cancer. *N Engl J Med* 2005, 353:229–237
17. Hartmann LC, Degnim AC, Santen RJ, Dupont WD, Ghosh K: Atypical hyperplasia of the breast: risk assessment and management options. *N Engl J Med* 2015, 372:78–89
18. Hartmann LC, Radisky DC, Frost MH, Santen RJ, Vierkant RA, Benetti LL, Tarabishy Y, Ghosh K, Visscher DW, Degnim AC: Understanding the premalignant potential of atypical hyperplasia through its natural history: a longitudinal cohort study. *Cancer Prev Res (Phila)* 2014, 7:211–217
19. Page DL, Schuyler PA, Dupont WD, Jensen RA, Plummer WD, Simpson JF: Atypical lobular hyperplasia as a unilateral predictor of breast cancer risk: a retrospective cohort study. *Lancet* 2003, 361:125–129
20. Thompson PA, DeMarini DM, Kadlubar FF, McClure GY, Brooks LR, Green BL, Fares MY, Stone A, Josephy PD, Ambrosone CB: Evidence for the presence of mutagenic arylamines in human breast milk and DNA adducts in exfoliated breast ductal epithelial cells. *Environ Mol Mutagen* 2002, 39:134–142
21. Petrakis NL, Gruenke LD, Beelen TC, Castagnoli N, Craig JC: Nicotine in breast fluid of nonlactating women. *Science* 1978, 199:303–305
22. Dasgupta P, Rizwani W, Pillai S, Kinkade R, Kovacs M, Rastogi S, Banerjee S, Carless M, Kim E, Coppola D, Haura E, Chellappan S: Nicotine induces cell proliferation, invasion and epithelial-mesenchymal transition in a variety of human cancer cell lines. *Int J Cancer* 2009, 124:36–45
23. Kispert S, Marentette J, McHowat J: Cigarette smoke induces cell motility via platelet-activating factor accumulation in breast cancer cells: a potential mechanism for metastatic disease. *Physiol Rep* 2015, 3:pii:e12318
24. Moinfar F, Man YG, Arnould L, Bratthauer GL, Ratschek M, Tavassoli FA: Concurrent and independent genetic alterations in the stromal and epithelial cells of mammary carcinoma: implications for tumorigenesis. *Cancer Res* 2000, 60:2562–2566
25. Boyd NF, Dite GS, Stone J, Gunasekara A, English DR, McCredie MR, Giles GG, Trichler D, Chiarelli A, Yaffe MJ, Hopper JL: Heritability of mammographic density, a risk factor for breast cancer. *N Engl J Med* 2002, 347:886–894
26. Ursin G, Hovanesian-Larsen L, Parisky YR, Pike MC, Wu AH: Greatly increased occurrence of breast cancers in areas of mammographically dense tissue. *Breast Cancer Res* 2005, 7:R605–R608
27. Provenzano PP, Inman DR, Eliceiri KW, Knittel JG, Yan L, Rueden CT, White JG, Keely PJ: Collagen density promotes mammary tumor initiation and progression. *BMC Med* 2008, 6:11
28. Preston-Martin S, Pike MC, Ross RK, Jones PA, Henderson BE: Increased cell division as a cause of human cancer. *Cancer Res* 1990, 50:7415–7421
29. Boesiger J, Tsai M, Maurer M, Yamaguchi M, Brown LF, Claffey KP, Dvorak HF, Galli SJ: Mast cells can secrete vascular permeability factor/vascular endothelial cell growth factor and exhibit enhanced release after immunoglobulin E-dependent upregulation of fc epsilon receptor I expression. *J Exp Med* 1998, 188:1135–1145
30. Möller A, Lippert U, Lessmann D, Kolde G, Hamann K, Welker P, Schadendorf D, Rosenbach T, Luger T, Czarnetzki BM: Human mast cells produce IL-8. *J Immunol* 1993, 151:3261–3266
31. Ribatti D, Crivellato E: Immune cells and angiogenesis. *J Cell Mol Med* 2009, 13:2822–2833
32. Zhang SX, Wang JJ, Gao G, Parke K, Ma JX: Pigment epithelium-derived factor downregulates vascular endothelial growth factor (VEGF) expression and inhibits VEGF-VEGF receptor 2 binding in diabetic retinopathy. *J Mol Endocrinol* 2006, 37:1–12
33. Becerra SP, Notario V: The effects of PEDF on cancer biology: mechanisms of action and therapeutic potential. *Nat Rev Cancer* 2013, 13:258–271
34. Ghosh A, Pechota A, Coleman D, Upchurch GR, Eliason JL: Cigarette smoke-induced MMP2 and MMP9 secretion from aortic vascular smooth cells is mediated via the Jak/Stat pathway. *Hum Pathol* 2015, 46:284–294
35. Ning W, Dong Y, Sun J, Li C, Matthy MA, Feghali-Bostwick CA, Choi AM: Cigarette smoke stimulates matrix metalloproteinase-2 activity via EGR-1 in human lung fibroblasts. *Am J Respir Cell Mol Biol* 2007, 36:480–490
36. Kim YH, Lee SJ, Seo KW, Bae JU, Park SY, Kim EK, Bae SS, Kim JH, Kim CD: PAF enhances MMP-2 production in rat aortic VSMCs via a β -arrestin2-dependent ERK signaling pathway. *J Lipid Res* 2013, 54:2678–2686
37. Melnikova VO, Mourad-Zeidan AA, Lev DC, Bar-Eli M: Platelet-activating factor mediates MMP-2 expression and activation via phosphorylation of cAMP-response element-binding protein and contributes to melanoma metastasis. *J Biol Chem* 2006, 281:2911–2922