

SV40, Growth Factors, and Mesothelioma Another Piece of the Puzzle

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The initiation and development of malignant mesothelioma, a tumor derived from mesothelial cells which line the pleural and peritoneal cavities, are under intense investigation as this unique tumor has been associated historically with occupational exposures to asbestos (1, 2), and is increasing in several countries (3). More importantly, the prognosis of patients with mesothelioma is grim, most surviving less than a year after initial diagnosis (1, 2). Thus, effective therapeutic strategies are desperately needed. In the past few years, simian virus 40 (SV40), a DNA virus, has been linked to the etiology of mesothelioma in multi-institutional studies showing that $\sim 50\%$ of human mesotheliomas in the United States contain SV40 large T antigen (T-Ag) DNA sequences (reviewed in Refs. 4, 5). The link between mesothelioma and SV40 appears to be related to the contamination of polio vaccine stocks with SV40 sequences, but it is unclear how the virus is spread in humans (4). In Finland, where the polio vaccine shows no SV40 contamination, the frequency of mesothelioma is significantly lower (10^{-5} compared with 1.4×10^{-5}) and associated with environmental asbestos exposure rather than concomitant exposure to SV40 sequences (6). A causal link between SV40 and mesothelioma has also been strengthened by studies showing that SV40 sequences are selectively expressed in mesothelioma cells, and not in adjacent parenchymal cells or lung carcinomas (7, 8). Moreover, mechanistic work demonstrates that human mesothelial cells are uniquely susceptible to SV40 infection and malignant transformation (9). In these experiments, infection of normal human mesothelial cells by SV40 led to an extremely high rate of morphologic transformation (at least 1,000 times higher than other cell types infected with this virus), and immortality. In addition, SV40 acts synergistically with asbestos to cause malignant transformation of human mesothelial cells (9).

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Abbreviations: Activator Protein-1, AP-1; epidermal growth factor, EGF; EGF receptor, EGFR; extracellular signal-regulated kinase, ERK; hepatocyte growth factor, HGF; human umbilical vein cells, HUVEC; interleukin, IL; keratinocyte growth factor, KGF; loss of heterozygosity, LOH; mitogen-activated protein kinase, MAPK; platelet-derived growth factor, PDGF; protein phosphatase 2A, PP2A; simian virus 40, SV40; small t antigen, t-Ag; large T antigen, T-Ag; transforming growth factor, TGF; tumor necrosis factor- α , TNF- α ; vascular endothelial growth factor, VEGF.

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SV40-associated mesothelial cell transformation has been attributed to the ability of SV40 T-Ag to inactivate the tumor suppressors, p53 (10) and p-retinoblastoma (Rb) family proteins (11). Inactivation of pRb and p53 by the SV40 T-Ag also has an indirect effect on p16 and p21 cyclin D-dependent kinases that appear to be depleted in mesothelioma cells as a result of loss of heterozygosity (LOH), and through a reduction in p53-dependent transcription, respectively (12, 13). As a result of the T-Ag activity, cyclin-dependent kinase subunits are rearranged (14). The SV40 small t-antigen (t-Ag) also modulates cell proliferation by enhancing the activity of telomerase (15), Extracellular Signal-Regulated Kinases (ERKs) and Activator Protein-1 (AP-1) (16). This appears to be a function of a t-Ag-mediated decrease in Protein Phosphatase 2A (PP2A).

Yet other events involved in immortalization of cells and tumor development by SV40 are clearly involved, because mesotheliomas have long latency periods in man, averaging from 25 to 40 yr (1). Growth factors may be critical stimuli of mesothelial cell proliferation, increasing not only the susceptibility of cells to DNA-damaging agents and genetic instability, but also the expansion of transformed cell populations. In addition, elaboration of growth factors may be essential to creation of a favorable environment for tumor development.

Normal human and rodent mesothelial cells proliferate in response to a number of growth factors, including epidermal growth factor (EGF) (17, 18), tumor necrosis factor (TNF)- α (17, 19), platelet-derived growth factor (PDGF) (18), hepatocyte growth factor (HGF) (20), and keratinocyte growth factor (KGF) (20). *In vivo*, rodent pleural mesothelial cells exhibit increases in DNA synthesis several days after inhalation (21) or intratracheal instillation of amphibole types of asbestos (20, 22). These early proliferative events may be mediated in part via increased levels of HGF and KGF in pleural lavage fluid (20), as opposed to direct effects of fibers on mesothelial cells, as few fibers have been detected at the pleural surface in this short time frame of exposure. Although HGF is produced in general by mesenchymal cells, recent work by Cacciotti and colleagues (23) shows that the HGF receptor, Met, a proto-oncogene product whose activation leads to cell growth and altered morphogenesis, is activated in SV40-positive mesothelioma cells. Moreover, when normal human mesothelial cells are transfected with full-length SV40 DNA, Met receptor activation is induced and associated with S-phase entry, fibroblastoid morphology, and the assembly of viral particles that infect adjacent mesothelial cells, inducing an HGF-dependent Met activation. This work

may be of special significance as high levels of HGF are detected in pleural effusions from patients with malignant mesothelioma (24). It also suggests a mechanism whereby SV40-infected cells may propagate infection and proliferation of surrounding mesothelial cells.

EGF is required for growth of normal human mesothelial cells (18, 25). Intriguingly, normal mesothelial cells transfected with the *EJras* oncogene become EGF-independent (26), a phenomenon not yet explored after infection with SV40. Although normal mesothelial cells, asbestos-transformed mesothelioma cells, and spontaneously transformed mesothelial cells express functional EGF receptors (EGFR) (19, 27), only cell lines derived from asbestos-induced mesotheliomas express and secrete transforming growth factor (TGF)- α , which binds to the EGF receptor with high affinity. In addition, TGF- α acts as an autocrine growth factor for asbestos-induced mesotheliomas, because their growth is inhibited with use of a neutralizing TGF- α antibody (27). TGF- β also plays a role in cell transformation and tumorigenesis (reviewed in Ref. 28), as antisense approaches inhibit growth of mesothelioma cells *in vivo* and *in vitro* (29). Interleukin (IL)-6 and IL-8 (30, 31) have also been implicated in reducing tumorigenesis in a nude mouse model.

PDGF may also be an autocrine growth factor for human mesothelioma cells as both PDGF-A and -B chain mRNAs are expressed at higher levels in mesothelioma as opposed to normal mesothelial cell lines, and PDGF-like mitogenic activity is observed using mesothelioma-cell line conditioned medium (32). Paradoxically, overexpression of PDGF-A has a growth inhibitory effect on human mesothelioma cells *in vitro*, but increases tumor incidence and growth rate after their injection into nude mice (33). Conversely, abrogation of PDGF through a knockdown antisense approach decreases the tumor incidence. These results support previous results showing that PDGF-A chain overexpression causes tumorigenic conversion of human mesothelial cells (34).

Insulin-like growth factors (IGF) I and II also function as autocrine growth factor stimuli in normal mesothelial and mesothelioma cells (35, 36), and release of IGF-1 is implicated in governing both growth rate and tumorigenicity of SV40-induced mesotheliomas (37). These results implicate multiple cytokine receptor-induced cell signaling cascades in the advent of mesothelial cell proliferation, and suggest that a focus on blocking common downstream events or points of convergence of these pathways may be merited in therapeutic strategies.

One common signaling cascade that is activated after phosphorylation of receptor tyrosine kinases is the ERK arm of the Mitogen-Activated Protein Kinases (MAPKs). This signaling pathway is associated with cell proliferation and survival and is uniquely stimulated, as opposed to the c-Jun-NH₂-terminal amino kinases (JNKs) and p38 kinases, in mesothelial (38, 39, 40) and pulmonary epithelial cells (41, 42) exposed to asbestos at concentrations causing early injury and subsequent proliferation of cells (17, 42). Phosphorylated ERK protein is also increased at sites of initial deposition of asbestos fibers in the lung at regions of epithelial cell proliferation (43). It is noteworthy that SV40 small t antigen (t-Ag) also stimulates ERK activity by

binding to and inhibiting Protein Phosphatase 2A, a protein involved in dephosphorylation of many protein substrates, including members of the MAPK pathway. This may have relevance to the carcinogenic process, as co-expression of SV40 T-Ag and t-Ag are required for SV40-mediated human cell transformation (44). Work in our laboratory has shown that ERK stimulation by asbestos fibers in mesothelial and epithelial cells is protracted and oxidant-dependent, a downstream event following phosphorylation and upregulation of the EGFR by asbestos fibers (38, 40, 45), and linked to subsequent expression of the protooncogenes and AP-1 family members, *c-fos* and *fra-1* (41). Moreover, inhibition of ERKs ameliorates both cell injury and subsequent proliferation after exposures to asbestos fibers (39, 40), suggesting that AP-1-dependent gene activation is causally involved.

The summary above suggests that multiple growth factors may be integral to mesothelial cell proliferation through autocrine and paracrine mechanisms. A new piece of the puzzle is revealed in the paper by Cacciotti and coworkers in this issue of the *American Journal of Respiratory Cell and Molecular Biology* (46). This work reveals that SV40 infection induces release of Vascular Endothelial Cell Growth Factor (VEGF) from human mesothelial cells. VEGF is not only an autocrine growth factor for human mesotheliomas (46), but a potent angiogenic factor necessary for vascularization and sustenance of tumors. In addition to showing that high levels of VEGF are produced by SV40 T antigen-positive mesothelioma cells, Cacciotti and colleagues demonstrate that normal human mesothelioma cells infected with a full length SV40 construct release high amounts of VEGF into medium. The functional ramifications are proven by induction, in co-culture experiments, of proliferation in Human Umbilical Vein Cells (HUVEC) which is abrogated by adding antibodies to VEGF. This work is intriguing as it illustrates for the first time that SV40 infection causes elaboration of a factor from human mesothelial cells that is critical to angiogenesis. The establishment of a favorable tumor environment may be relevant to both asbestos- and SV40-induced mesotheliomas and may be one mechanism whereby SV40 acts cooperatively with asbestos in the development of these malignancies. Coupled with asbestos-induced modifications in cell

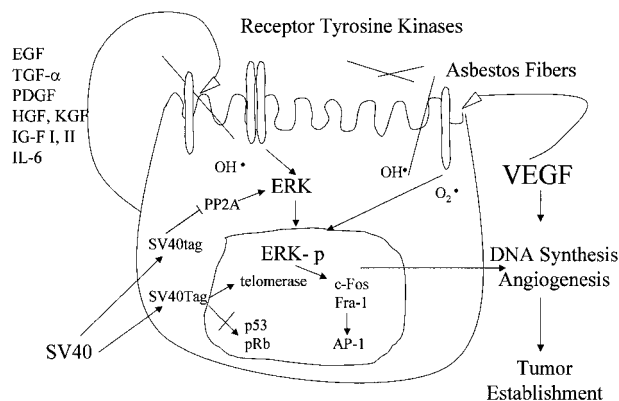


Figure 1. Growth factor elaboration and cell signaling pathways implicated in mesothelioma.

signaling and in enhancement of angiogenesis, cells expressing the SV40 T-Ag and t-Ag appear to be primed for proliferation and tumorigenesis.

Figure 1 shows a hypothetical schema of events that may govern cell proliferation and establishment of mesotheliomas. In this scenario, growth factors may act primarily through autocrine mechanisms to stimulate receptor tyrosine kinases and downstream signaling cascades, which then lead to activation of transcription factors, i.e., AP-1, etc., and induction of gene expression critical to cell proliferation. These events favor tumor promotion and progression. On the other hand, growth factors such as VEGF may also encourage vascularization of mesotheliomas in later stages of tumor establishment. Discerning and blocking the pathways of growth factor upregulation and secretion, as well as receptor activation, may be critical to effective strategies for prevention and therapy of mesotheliomas.

References

- Mossman, B. T., and J. B. Gee. 1989. Asbestos-related diseases. *N. Engl. J. Med.* 320:1721-1730.
- Mossman, B. T., J. Bignon, M. Corn, A. Seaton and J. B. Gee (1990). Asbestos: scientific developments and implications for public policy. *Science* 247:294-301.
- McDonald, J. C., and A. D. McDonald. 1996. The epidemiology of mesothelioma in historical context. *Eur. Respir. J.* 9:1932-1942.
- Klein, G., A. Powers, and C. Croce. 2002. Association of SV40 with human tumors. *Oncogene* (In press.)
- Pass, H. I., J. S. Donington, P. Wu, P. Rizzo, M. Nishimura, R. Kennedy, and M. Carbone. 1998. Human mesotheliomas contain the simian virus-40 regulatory region and large tumor antigen DNA sequences. *J. Thorac. Cardiovasc. Surg.* 116:854-859.
- Hirvonen, A., K. Mattson, A. Karjalainen, T. Ollikainen, L. Tammilehto, T. Hovi, H. Vainio, H. I. Pass, I. Di Resta, M. Carbone, and K. Linnainmaa. 1999. Simian virus 40 (SV40)-like DNA sequences not detectable in Finnish mesothelioma patients not exposed to SV40-contaminated polio vaccines. *Mol. Carcinog.* 26:93-99.
- Shivapurkar, N., T. Wiethege, I. I. Wistuba, E. Salomon, S. Milchgrub, K. M. Muller, A. Churg, H. Pass and A. F. Gazdar. 1999. Presence of simian virus 40 sequences in malignant mesotheliomas and mesothelial cell proliferations. *J. Cell Biochem.* 76:181-188.
- Toyooka, S., H. I. Pass, N. Shivapurkar, Y. Fukuyama, R. Maruyama, K. O. Toyooka, M. Gilcrease, A. Farinas, J. D. Minna, and A. F. Gazdar. 2001. Aberrant methylation and simian virus 40 tag sequences in malignant mesothelioma. *Cancer Res.* 61:5727-5730.
- Bocchetta, M., I. Di Resta, A. Powers, R. Fresco, A. Tosolini, J. R. Testa, H. I. Pass, P. Rizzo, and M. Carbone. 2000. Human mesothelial cells are unusually susceptible to simian virus 40-mediated transformation and asbestos cocarcinogenicity. *Proc. Natl. Acad. Sci. USA* 97:10214-10219.
- Carbone, M., P. Rizzo, P. M. Grimley, A. Procopio, D. J. Mew, V. Shridhar, A. de Bartolomeis, V. Esposito, M. T. Giuliano, S. M. Steinberg, A. S. Levine, A. Giordano, and H. I. Pass. 1997. Simian virus-40 large-T antigen binds p53 in human mesotheliomas. *Nat. Med.* 3:908-912.
- De Luca, A., A. Baldi, V. Esposito, C. M. Howard, L. Bagella, P. Rizzo, M. Caputi, H. I. Pass, G. G. Giordano, F. Baldi, M. Carbone, and A. Giordano. 1997. The retinoblastoma gene family pRb/p105, p107, pRb2/p130 and simian virus-40 large T-antigen in human mesotheliomas. *Nat. Med.* 3:913-916.
- Hara, E., R. Smith, D. Parry, H. Tahara, S. Stone, and G. Peters. 1996. Regulation of p16CDKN2 expression and its implications for cell immortalization and senescence. *Mol. Cell. Biol.* 16:859-867.
- Murthy, S. S., and J. R. Testa. 1999. Asbestos, chromosomal deletions, and tumor suppressor gene alterations in human malignant mesothelioma. *J. Cell. Physiol.* 180:150-157.
- Xiong, Y., H. Zhang, and D. Beach. 1993. Subunit rearrangement of the cyclin-dependent kinases is associated with cellular transformation. *Genes Dev.* 7:1572-1583.
- Foddiss, R., A. D. Rienzo, D. Broccoli, M. Bocchetta, E. Stekala, P. Rizzo, A. Tosolini, J. V. Grobely, S. C. Jhanwar, H. I. Pass, J. R. Testa, and M. Carbone. 2002. SV40 infection induces telomerase activity in human mesothelial cells. *Oncogene* (In press.)
- Carbone, M., P. Rizzo, and H. I. Pass. 1997. Simian virus 40, poliovaccines and human tumors: a review of recent developments. *Oncogene* 15:1877-1888.
- Goldberg, J. L., C. L. Zanella, Y. M. Janssen, C. R. Timblin, L. A. Jimenez, P. Vacek, D. J. Taatjes, and B. T. Mossman. 1997. Novel cell imaging techniques show induction of apoptosis and proliferation in mesothelial cells by asbestos. *Am. J. Respir. Cell Mol. Biol.* 17:265-271.
- Gabrielson, E. W., J. F. Lechner, B. I. Gerwin, M. A. Laveck, and C. C. Harris. 1987. Growth factors for mesothelial and mesothelioma cells. *Chest* 91:17S-18S.
- Bermudez, E., J. Everitt, and C. Walker. 1990. Expression of growth factor and growth factor receptor RNA in rat pleural mesothelial cells in culture. *Exp. Cell Res.* 190:91-98.
- Adamson, I. Y., and J. Bakowska. 2001. KGF and HGF are growth factors for mesothelial cells in pleural lavage fluid after intratracheal asbestos. *Exp. Lung Res.* 27:605-616.
- BeruBe, K. A., T. R. Quinlan, G. Moulton, D. Hemenway, P. O'Shaughnessy, P. Vacek, and B. T. Mossman. 1996. Comparative proliferative and histopathologic changes in rat lungs after inhalation of chrysotile or crocidolite asbestos. *Toxicol. Appl. Pharmacol.* 137:67-74.
- Sekhon, H., J. Wright, and A. Churg. 1995. Effects of cigarette smoke and asbestos on airway, vascular and mesothelial cell proliferation. *Int. J. Exp. Pathol.* 76:411-418.
- Cacciotti, P., R. Libener, P. Betta, F. Martini, C. Porta, A. Procopio, L. Strizzi, L. Penengo, M. Tognon, L. Mutti, and G. Gaudino. 2001. SV40 replication in human mesothelial cells induces HGF/Met receptor activation: a model for viral-related carcinogenesis of human malignant mesothelioma. *Proc. Natl. Acad. Sci. USA* 98:12032-12037.
- Eagles, G., A. Warn, R. Y. Ball, H. Baillie-Johnson, N. Arakaki, Y. Daikuhara, and R. M. Warn. 1996. Hepatocyte growth factor/scatter factor is present in most pleural effusion fluids from cancer patients. *Br. J. Cancer* 73:377-381.
- Laveck, M. A., A. N. Somers, L. L. Moore, B. I. Gerwin, and J. F. Lechner. 1988. Dissimilar peptide growth factors can induce normal human mesothelial cell multiplication. *In Vitro Cell. Dev. Biol.* 24:1077-1084.
- Tube, R. A., and J. G. Rheinwald. 1987. Normal human mesothelial cells and fibroblasts transfected with the *EJras* oncogene become EGF-independent, but are not malignantly transformed. *Oncogene Res.* 1:407-421.
- Walker, C., J. Everitt, P. C. Ferriola, W. Stewart, J. Mangum, and E. Bermudez. 1995. Autocrine growth stimulation by transforming growth factor α in asbestos-transformed rat mesothelial cells. *Cancer Res.* 55:530-536.
- Upham, J. W., M. J. Garlepp, A. W. Musk, and B. W. Robinson. 1995. Malignant mesothelioma: new insights into tumour biology and immunology as a basis for new treatment approaches. *Thorax* 50:887-893.
- Marzo, A. L., D. R. Fitzpatrick, B. W. Robinson, and B. Scott. 1997. Antisense oligonucleotides specific for transforming growth factor beta2 inhibit the growth of malignant mesothelioma both *in vitro* and *in vivo*. *Cancer Res.* 57:3200-3207.
- Fujino, S., A. Yokoyama, N. Kohno, and K. Hiwada. 1996. Interleukin 6 is an autocrine growth factor for normal human pleural mesothelial cells. *Am. J. Respir. Cell Mol. Biol.* 14:508-515.
- Galfy, G., K. A. Mohammed, P. A. Dowling, N. Nasreen, M. J. Ward, and V. B. Antony. 1999. Interleukin 8: an autocrine growth factor for malignant mesothelioma. *Cancer Res.* 59:367-371.
- Gerwin, B. I., J. F. Lechner, R. R. Reddel, A. B. Roberts, K. C. Robbins, E. W. Gabrielson, and C. C. Harris. 1987. Comparison of production of transforming growth factor- β and platelet-derived growth factor by normal human mesothelial cells and mesothelioma cell lines. *Cancer Res.* 47:6180-6184.
- Metheny-Barlow, L. J., B. Flynn, H. E. van Gijssel, A. Marrogi, and B. I. Gerwin. 2001. Paradoxical effects of platelet-derived growth factor-A overexpression in malignant mesothelioma: antiproliferative effects *in vitro* and tumorigenic stimulation *in vivo*. *Am. J. Respir. Cell Mol. Biol.* 24:694-702.
- Van der Meer, A., M. B. Seddon, C. A. Betsholtz, J. F. Lechner, and B. I. Gerwin. 1993. Tumorigenic conversion of human mesothelial cells as a consequence of platelet-derived growth factor-A chain overexpression. *Am. J. Respir. Cell Mol. Biol.* 8:214-221.
- Lee, T. C., Y. Zhang, C. Aston, R. Hintz, J. Jagirdar, M. A. Perle, M. Burt, and W. N. Rom. 1993. Normal human mesothelial cells and mesothelioma cell lines express insulin-like growth factor I and associated molecules. *Cancer Res.* 53:2858-2864.
- Rutten, A. A., E. Bermudez, W. Stewart, J. I. Everitt, and C. L. Walker. 1995. Expression of insulin-like growth factor II in spontaneously immortalized rat mesothelial and spontaneous mesothelioma cells: a potential autocrine role of insulin-like growth factor II. *Cancer Res.* 55:3634-3639.
- Pass, H. I., D. J. Mew, M. Carbone, W. A. Matthews, J. S. Donington, R. Baserga, C. L. Walker, M. Resnicoff, and S. M. Steinberg. 1996. Inhibition of hamster mesothelioma tumorigenesis by an antisense expression plasmid to the insulin-like growth factor-1 receptor. *Cancer Res.* 56:4044-4048.
- Zanella, C. L., J. Posada, T. R. Tritton, and B. T. Mossman. 1996. Asbestos causes stimulation of the extracellular signal-regulated kinase 1 mitogen-activated protein kinase cascade after phosphorylation of the epidermal growth factor receptor. *Cancer Res.* 56:5334-5338.
- Jimenez, L. A., C. Zanella, H. Fung, Y. M. Janssen, P. Vacek, C. Charland, J. Goldberg, and B. T. Mossman. 1997. Role of extracellular signal-regulated protein kinases in apoptosis by asbestos and H₂O₂. *Am. J. Physiol.* 273(5, Pt. 1):L1029-L1035.
- Zanella, C. L., C. R. Timblin, A. Cummins, M. Jung, J. Goldberg, R. Raabe,

- T. R. Tritton, and B. T. Mossman. 1999. Asbestos-induced phosphorylation of epidermal growth factor receptor is linked to *c-fos* and apoptosis. *Am. J. Physiol.* 277(4, Pt. 1):L684–L693.
41. Shukla, A., C. R. Timblin, A. K. Hubbard, J. Bravman, and B. T. Mossman. 2001. Silica-induced activation of c-Jun-NH₂-terminal amino kinases, protracted expression of the activator protein-1 proto-oncogene, *fra-1*, and S-phase alterations are mediated via oxidative stress. *Cancer Res.* 61:1791–1795.
42. Buder-Hoffmann, S., C. Palmer, P. Vacek, D. Taatjes, and B. Mossman. 2001. Different accumulation of activated extracellular signal-regulated kinases (ERK 1/2) and role in cell-cycle alterations by epidermal growth factor, hydrogen peroxide, or asbestos in pulmonary epithelial cells. *Am. J. Respir. Cell Mol. Biol.* 24:405–413.
43. Robledo, R. F., S. A. Buder-Hoffmann, A. B. Cummins, E. S. Walsh, D. J. Taatjes, and B. T. Mossman. 2000. Increased phosphorylated extracellular signal-regulated kinase immunoreactivity associated with proliferative and morphologic lung alterations after chrysotile asbestos inhalation in mice. *Am. J. Pathol.* 156:1307–1316.
44. Rundell, K., and R. Parakati. 2001. The role of the SV40 ST antigen in cell growth promotion and transformation. *Semin. Cancer Biol.* 11:5–13.
45. Pache, J. C., Y. M. Janssen, E. S. Walsh, T. R. Quinlan, C. L. Zanella, R. B. Low, D. J. Taatjes, and B. T. Mossman. 1998. Increased epidermal growth factor-receptor protein in a human mesothelial cell line in response to long asbestos fibers. *Am. J. Pathol.* 152:333–340.
46. Cacciotti, P., L. Strizzi, G. Vianale, L. Iaccheri, R. Libener, C. Porta, M. Tognon, G. Gaudino, and L. Mutti. 2002. The presence of simian-virus 40 sequences in mesothelioma and mesothelial cells is associated with high levels of vascular endothelial growth factor. *Am. J. Respir. Cell Mol. Biol.* 26:189–193.