Journal of Cellular Biochemistry Supplement 34:61–66 (2000)

Differential Expression of Genes by Tumor Cells of a Low or a High Malignancy Phenotype: The Case of Murine and Human Ly-6 Proteins

Isaac P. Witz

Department of Cell Research and Immunology, George S. Wise Faculty of Life Sciences, Tel Aviv University, 69978 Tel Aviv, Israel

Abstract Progression of tumor cells toward a high malignancy phenotype and metastasis is a multi-event cascade involving inter alia alterations in the expression of various genes. The focus of our laboratory is on genes whose altered expression may lead, directly or indirectly, to an increased malignancy phenotype. The identification of such genes and the evaluation of the consequences of their altered expression is essential for attempts to halt tumor progression and prevent metastasis formation. Published work originating in our laboratory showed that members of the murine Ly-6 supergene family are involved in the progression of certain mouse tumors. The expression level of several members of this family was higher on highly malignant cells than on tumor cells expressing a lower malignancy phenotype. Sorting by flow cytometry of tumor cells to subpopulations expressing either high or low levels of Ly-6E.1 yielded correspondingly cells expressing a high or a low malignancy phenotype. The high malignancy, high Ly-6E.1-expressing cells also expressed high levels of the receptor for urokinase plasminogen activator (uPAR), whereas low malignancy, low Ly-6E.1-expressing cells also expressed low levels of uPAR. Transfection studies indicated that uPAR was causally involved in conferring a high malignancy phenotype upon tumor cells expressing high levels of Ly-6E.1. E48 is a human homologue of the murine ThB protein, a member of the Ly-6 supergene family (but distinct from the Ly-6E.1 protein mentioned above) and expressed on head and neck squamous carcinoma cells. Experiments currently in progress are aimed to find out whether E48 is involved in the progression of such cancer cells. Using the differential display technology, it was shown that ligation of E48 on tumor cells by the corresponding antibodies (serving as a surrogate for an as yet unidentified E48 ligand) upregulates an enzyme (FX) involved in the biosynthesis of GDP-L-fucose. Fucose is an essential component of certain selectin ligands. J. Cell. Biochem. Suppl. 34:61–66, 2000.

Key words: tumor progression; Ly-6; uPAR; gene expression

Various types of alterations in oncogenes and in tumor suppressor genes are responsible for the key events of carcinogenesis [Kinzler and Vogelstein, 1996]. In addition to these alterations, numerous genes (that cannot, at least as yet, be defined as oncogenes or tumor suppresser genes) are differentially expressed in cancer cells and in normal cells. In a recent analysis using the newly developed method of serial analysis of gene expression it was shown that about 1.5% of genes are differentially expressed in normal and cancerous colorectal epithelium [Zhang et al., 1997]. It is not unreasonable to assume that at least some of these differentially expressed genes contribute positively or negatively to local tumorigenicity and tumor progression. A comprehensive comparison in gene expression profiles of nonmetastatic and metastatic tumor cells has not been constructed as yet. However, numerous studies focusing on certain gene products that are likely to play a role in tumor progression toward metastasis have demonstrated differences in their expression between less and more progressed tumor cells [Zhang et al., 1997; Zusman

Grant sponsor: U.S.-Israel Binational Science Foundation; Grant sponsor: German-Israeli Foundation for Scientific Research and Development (GIF); Grant sponsor: Cooperation Program in Cancer Research of the Deutsches Krebsforschungszentrum (DKFZ); Grant sponsor: Israel's Ministry of Science; Grant sponsor: Fainbarg Family Fund, Orange County, CA; Grant sponsor: Pikovsky Fund, Jerusalem, Israel.

I.P. Witz is the incumbent of the David Furman Chair in Immunobiology of Cancer.

Correspondence to: Isaac P. Witz, Department of Cell Research and Immunology, George S. Wise Faculty of Life Sciences, Tel Aviv University, 69978 Tel Aviv, Israel.

Received 12 August 1998; Accepted 12 August 1998

et al., 1996a; Katz et al., 1994; Fidler et al., 1996; Witz et al., 1996]. Because tumor progression toward metastasis largely involves genes that control the social behavior of cancer cells such as adhesion, motility, invasiveness, and interaction with growth factors [Hakomori, 1996], these genes should be focused on. Numerous studies showed that factors present in the tumor microenvironment are able to regulate the expression of such progression-associated genes. Cancer-microenvironmental factors (CMF) may behave as a double-edged sword exerting opposing (cancer progression enhancing or cancer progression restraining) effects. This bidirectionality is illustrated by 3 examples: Tumor infiltrating immunocytes or tumor-localized immunoglobulins may positively or negatively correlate to tumorigenesis and tumor progression [Witz, 1977]. Certain cytokines are powerful anti-tumor agents being already utilized in clinical trials while conversely, these or other cytokines may promote and enhance metastasis [Croxtall et al., 1992; Ramani and Balkwill, 1987]. Laminin, a major component of the extracellular matrix, contains peptides that may either suppress or conversely enhance metastasis [Kibbey et al., 1992; Yamamura et al., 1993].

Therefore, it would appear highly logical to attempt to strengthen tumor-CMF interactions that destroy or inhibit tumor cells and to block metastasis-promoting tumor-CMF interactions. However, the spectrum of tumor-CMF interactions is far from being completely evaluated, and the significance of many such interactions remains to be elucidated [Witz et al., 1996; Nicolson, 1991; Radinsky and Fidler, 1992; Fidler, 1995].

This mini-review illustrates the complexity of tumor CMF interactions. One family of proteins that is differentially expressed by low and high malignancy tumor cells of human and mouse origin is described and their role in biochemical cascades leading to a high malignancy phenotype evaluated. This gene family and/or the genes regulating their expression could conceivably be targeted in order to prevent or eradicate metastasis.

Microenvironment-Regulated Genes and the Involvement of Murine Ly-6 Proteins in Tumor Progression

The work in our laboratory focuses on tumor-CMF interactions trying to identify genes whose expression is altered (induced/upregulated or silenced/downregulated) by CMF-mediated signaling as low malignancy tumor cells progress to a high malignancy phenotype [Zusman et al., 1996a; Katz et al., 1994, 1994–1995; Witz et al., 1996; Halachmi and Witz, 1989; Ben-Baruch et al., 1992; Ran et al., 1991]. The approach undertaken has been to screen for microenvironment-regulated gene-products differentially expressed by tumor cells expressing either a high or a low malignancy phenotype [Katz et al., 1994; Witz et al., 1996; Halachmi and Witz, 1989]. By using a combined in vitro/in vivo murine model system developed in our laboratory [Halachmi and Witz, 1989], we were the first to show that certain highly tumorigenic murine tumor cells express de novo receptors for the Fc portion of IgG (FcγRIIB1), whereas low tumorigenic tumor cells did not [Zusman et al., 1996a,b; Witz et al., 1996; Ben-Baruch et al., 1992; Ran et al., 1991]. Gene transfer experiments indicated that FcγRIIB1 was causally involved in enhancing the tumorigenicity phenotype of the tumor cells and that the cytoplasmic domain of the receptor was the key player in this enhancement [Zusman et al., 1996a,b].

Other gene products found by us to be differentially expressed by murine tumor cells differing in their local tumorigenicity and metastatic phenotype are members of the Ly-6 supergene family [for reviews, see Shevach and Korty, 1989; Rock et al., 1989; Gumley et al., 1995]. Fibroblastic tumor cells and mammary adenocarcinoma cells were sorted by flow cytometry into stable subpopulations differing in the expression of Ly-6E.1 and of Ly-6C.1 and Ly-6G.1 [Katz et al., 1994; Treister et al., 1998]. High Ly-6 (Ly-6^{hi}) expressors in both tumor systems were significantly more tumorigenic and metastatic than low Ly-6-expressing (Ly-6^{lo}) tumor cells [Katz et al., 1994; Treister et al., 1998]. These results were confirmed by other investigators [Cohn et al., 1996]. Comparative karyotyping of Ly-6^{hi} and Ly-6^{lo} tumor cells carried out by Itshak Golan from our laboratory revealed that both showed a chromosome 15 trisomy. There was however no difference in this respect between the two types of tumor cells. The results of fluorescent in situ hybridization (FISH) experiments as well as Southern blot analysis ruled out an amplification of the Ly-6E.1 gene in Ly-6^{hi} cells. By contrast, Northern blot analysis and run-on assays indicated an increased transcriptional activity of Ly-6E.1 in

Ly-6^{hi} tumor cells. It was also found that Ly-6 mRNA stability in Ly-6^{hi} tumor cells was higher than that in Ly-6^{lo} cells (unpublished).

Membrane Ly-6 levels on tumor cells were upregulated by interferon α (IFNα) and by coating such cells with monoclonal antibodies directed against Ly-6E.1 [Eshel et al., 1995]. Ly-6E.1 levels were slightly downregulated by tumor necrosis factor-α (TNFα) and drastically downregulated by anti Fas antibodies [Sagi-Assif et al., 1996]. Serum starvation and heat shock significantly upregulated Ly-6 levels expressed by the tumor cells. The stress-mediated effects on Ly-6 levels were less pronounced in the mammary carcinoma cells than in the fibroblastic tumors [Treister et al., 1998].

In order to determine whether the high expression of Ly-6 is causally related to a high malignancy phenotype, gene transfer experiments were performed by Itshak Golan from our laboratory, with the collaboration of Ethan Shevach from the NIH. Ly-6^{lo} mammary tumor cells were transfected with Ly-6E.1 cDNA and Ly-6^{hi} tumor cells were transfected with Ly6-E.1 antisense. High malignancy tumor cells remained highly malignant even after Ly-6E.1 levels were decreased by Ly-6 antisense. Low malignancy tumor cells retained their low malignancy phenotype even after their Ly-6 expression was increased after transfection with Ly6-E.1 cDNA. We tentatively conclude that Ly-6E.1 is not involved, as a sole factor, in shaping the malignancy phenotype of these mammary adenocarcinoma cells (submitted for publication). These studies suggest that one or more cofactors, perhaps coregulated and functioning in conjunction with Ly-6E.1, may confer a high malignancy phenotype upon the tumor cells. Tsipora Meshel, Itshak Golan, and Orit Sagi-Assif from our laboratory, again in collaboration with Ethan Shevach, tested wheher the receptor for urokinase plasminogen activator (uPAR) is coregulated with Ly-6E.1. uPAR was studied because this molecule is also a GPI-linked protein being structurally related to the Ly-6 family and because uPAR was shown to be involved in tumor and metastasis formation [Andreasen et al., 1997]. Northern blotting analyses of RNA preparations from Ly-6^{hi} and Ly-6^{lo} DA3 tumor cells showed that the expression of uPAR mRNA is significantly higher in Ly-6^{hi} than in Ly-6^{lo} cells (manuscript in preparation). These results suggest that uPAR may be coregulated with Ly-6E.1 in tumor cells. In order to find out whether uPAR is a cofactor involved in conferring a high malignancy phenotype upon Ly-6^{hi} tumor cells, a vector carrying uPAR antisense (a.s.) was transfected to highly tumorigenic and metastatic Ly-6^{hi} DA3 cells. Control cells were transfected with empty vector. uPAR a.s. and control transfectants were inoculated into untreated BALB/c mice. The results showed that uPAR a.s. transfection was capable of reducing the local tumorigenicity and spontaneous pulmonary load of the mammary tumor cells (manuscript in preparation). This experiment also suggests that uPAR, perhaps together with Ly-6, functions to increase the malignancy and metastatic phenotype of tumor cells. An identical conclusion was derived from results of the reverse experiment in which uPAR cDNA was transfected into Ly-6^{lo} DA3 tumor cells expressing a low malignancy phenotype. This transfection significantly increased the malignancy phenotype of the transfectants (submitted for publication).

The next set of experiments was aimed to demonstrate a possible link between an angiogenic phenotype of tumor cells and Ly-6 expression. After an i.p. inoculation of cloned fibroblastic tumor cells to syngeneic mice, highly angiogenic (ang^{+}) and poorly angiogenic (ang^{-}) tumors were obtained: These tumors were introduced separately into culture. The cultured ang$^{(+)}$ cells were more highly tumorigenic and metastatic than ang$^{(-)}$ cells [Sagi-Assif et al., 1996]. The cells were also analyzed for Ly-6E.1 expression. It was found that the ang$^{(+)}$ cells expressed considerably higher levels of Ly-6E.1 than the ang$^{(-)}$ cells [Sagi-Assif et al., 1996].

Eran Neumark, in collaboration with Dr. Adit Ben Baruch and R. Anavi from our department, compared the ability of Ly-6^{hi} and Ly-6^{lo} DA3 cells to secrete chemotactic factors for monocytes using modified Boyden chemotaxis chambers. The results of these preliminary experiments showed that the former cells secrete considerably more monocytes chemoreactants than the latter ones. The use of inhibitory antibodies showed that the chemokine exerting the chemotactic function was identified as MCP-1 [Neumark et al., 1999]. The fact that Ly-6^{hi} cells express a higher angiogenic phenotype than Ly-6^{lo} cells, that Ly-6^{hi} cells express higher levels of uPAR than Ly-6^{lo} cells, and that Ly-6^{hi} cells secrete more MCP-1 than Ly-6^{lo} cells may be linked to a single mechanism based on the following working hypothesis.

Ly-6^{hi} DA3 cells secrete more MCP-1 than do Ly-6^{lo} DA3 cells and thus attract more monocytes and macrophages into the tumor site. The fact that such infiltrating cells secrete angiogenic factors [Leek et al., 1996] may explain the observation that cells derived from angiogenic tumors express higher levels of Ly-6 than cells derived from poorly angiogenic tumors. Furthermore, uPAR and its ligand play a role in tumor angiogenesis possibly by concentrating angiogenic factors at the membrane of tumor cells [Andreasen et al., 1997]. The higher expression of uPAR by Ly-6^{hi} cells may thus cause a high localization of angiogenic uPAR ligands on these cells.

Does E48, a Human Ly-6 Protein, Play a Role in the Progression on Cancer Cells?

In view of the involvement of Ly-6 in the progression of mouse tumors, we decided to examine whether these studies could be transcribed to human cancer. Preliminary studies carried out with E48, the first human Ly-6 gene to be identified and cloned (out of three cloned thus far)[Brakenhoff et al., 1995; Mao et al., 1996; Kimura et al., 1997], suggest that E48 may indeed be involved in the shaping of the malignancy phenotype of certain cancer cells.

The E48 monoclonal antibody (MAb) was one of several MAbs developed in order to detect and treat minimal residual disease of head and neck squamous carcinoma cells (HNSCC) after primary treatment [Quak et al., 1990]. The MAb E48 recognizes an outer membrane antigen which was expressed by the majority of HNSCC. Biodistribution of the E48 MAb in HNSCC patients showed that radioimmunotherapy using this antibody as a targeting vehicle was a feasible approach for the eradication of small tumor deposits [De Bree et al., 1995]. The antigen recognized the MAb E48 was chemically isolated and its cDNA cloned [Brakenhoff et al., 1995]. It proved to be a glycosyl-phosphatidylinositol (GPI) anchored membrane protein expressed by squamous cells. E48 is highly (nearly 70%) homologous to the murine ThB protein, a member of the Ly-6 gene family [Gumley et al., 1995; Brakenhoff et al., 1995]. The nucleotide sequence of ThB shows only a low (about 25%) degree of homology to the Ly-6 proteins (Ly-6E.1; Ly-6C.1 and Ly-6G.1) described above. E48 maps to human chromosome 8q24 [Brakenhoff et al., 1995], which is the syntenic locus of mouse chromosome 15E, the location of the mouse Ly-6 gene family [Shevach and Korty, 1989]. Whereas both E48 and ThB are expressed on keratinocytes of squamous epithelia [Brakenhoff et al., 1995], they differ in lymphocyte expression. ThB is expressed by mouse lymphocytes, but E48 is not expressed by human lymphocytes. The function of E48 on keratinocytes of squamous epithelia is still unclear although it seems to be involved in cellular adhesion [Brakenhoff et al., 1995]. Shortly after the cloning of E48, two additional human Ly-6 genes were cloned [Mao et al., 1996; Kimura et al., 1997].

Preliminary results obtained in our laboratory indicate that, in addition to its involvement in adhesion, E48is capable of transducing signals to squamous carcinoma cells. The downstream effects of these signals are manifested by the induction/ upregulation (or perhaps also silencing/ downregulation) of genes that may play a role in tumorigenesis and tumor progression.

In studies performed by Rinat Eshel in our laboratory, in collaboration with Drs. Brakenhoff and van Dongen from the Free University of Amsterdam, we set out todetermine whether E48 is capable of altering the expression of cellular genes in HNSCC by transducing signals to such cells. MAbs against E48 were added to HNSCC as a surrogate ligand (the physiological ligand of E48 has not been identified thus far). The method used to detect altered gene expression was differential display (DD) polymerase chain reaction PCR of mRNA [Liang and Pardee, 1992]. A higher expression of two mRNA species corresponding to the two cDNA species identified in the DD assays was displayed using Northern blotting of RNA from untreated or antibody-treated HNSCC. One of the corresponding cDNA species was then cloned and sequenced. Genebank analysis showed that it had a 98.2% homology to FX, a homodimeric nicotinamide adenine dinucleotide phosphate [NADP(H)]-binding protein of 68 kD first identified in human erythrocytes about 20 years ago [De Flora et al., 1977]. FX has been recently identified as the enzyme responsible for the last step of the major metabolic pathway resulting in guanosine 5′-diphosphate (GDP) L-fucose synthesis from GDP-D-mannose [Tonetti et al,. 1996]. GDP-L-fucose is the substrate of several fucosyl transferases involved in the correct ex-

pression of many glycoconjugates including blood group components and tumor-associated antigens. Fucosylated glycoconjugates function as ligands for selectins—a family of cell adhesion receptors with lectin-like N-terminal domains [Springer and Lasky, 1991]. The interactions of selectins with their ligands play highly significant roles in developmental and physiological processes, as well as in tumor progression and metastasis [Springer and Lasky, 1991; Sun et al., 1995; Walz et al., 1990; Kong et al., 1993]. Experiments in progress in our laboratory are aimed to find out whether the E48-mediated upregulation of FX bears consequences with respect to selectin-ligand expression on HNSCC and whether it facilitates interaction of the tumor cells with endothelial cells.

CONCLUDING REMARKS

Although the progression of tumor cells toward high malignancy and metastasis is accompanied by mutations or deletions of certain genes in cancer cells [Kinzler and Vogelstein, 1996], many of the properties of premetastatic cells and their progression toward metastasis are determined by an aberrant regulation of unaltered genes [Sager, 1997; Witz et al., 1996; Nicolson, 1991] governing primarily the social behavior of cells [Hakomori, 1996]. In order to design intelligent and rational approaches to halt tumor progression and prevent or delay metastasis formation, it is imperative that the molecules regulating the expression of such genes be identified and the cascades driving tumor progression be fully elucidated.

REFERENCES

Andreasen PA, Kjøller L, Christensen L, Duffy MJ. 1997. The urokinase-type plasminogen activator system in cancer metastasis: a review. Int J Cancer 72:1–22.

Ben-Baruch Langer A, Emmanuel N, Even J, Fridman WH, Gohar O, Gonen B, Katz B-Z, Ran M, Smorodinsky NI, Witz IP. 1992. Phenotypic properties of 3T3 cells transformed in vitro with polyoma virus and passaged once in syngeneic animals. Immunobiology 185:281–291.

Brakenhoff RH, Gerretsen M, Knippels PMC, Van Dijk M, Van Essen H, Olde Weghuis D, Sinke RJ, Snow GB, Van Dongen GAMS. 1995. The human E48 antigen, highly homologous with the murine Ly-6 antigen ThB, is a GPI-anchored molecule apparently involved in keratinocyte cell-cell adhesion. J Cell Biol 129:1677–1689.

Cohn MA, Kramerov D, Hulgaard EF, Lukanidin EM. 1997. The differentiation antigen Ly-6E.1 is expressed in mouse metastatic tumor cell lines. FEBS Lett 403:181–185.

Croxtall JD, Jamil A, Ayub M, Colletta AA, White JO. 1992. TGF-β stimulation of endometrial and breast-cancer cell growth. Int J Cancer 50:822–827.

De Bree R, Roos JC, Quak JJ, Den Hollander W, Wilhelm AJ, Van Lingen A, Snow GB, Van Dongen GAMS. 1995. Biodistribution and pharmacokinetics of radiolabeled monoclonal antibodies E48 IgG and $F(ab)_2$ in patients with head and neck cancer. Clin Cancer Res 1:277–286.

De Flora A, Morelli A, Frascio M, Corte G, Curti B, Galliano M, Gozzer C, Minchiotti L, Mareni C, Gaetani G. 1977. Radioimmunoassay and chemical properties of glucose-6-phosphate dehydrogenase and of a specific NADP(H)-binding protein (FX) from human erythrocytes. Biochim Biophys Acta 500:109–123.

Eshel R, Firon M, Katz B-Z, Sagi-Assif O, Aviram H, Witz IP. 1995. Microenvironmental factors regulate Ly-6 A/E expression on PyV-transformed BALB/c 3T3 cells. Immunol Lett 44:209–212.

Fidler IJ. 1995. Modulation of the organ microenvironment for the treatment of cancer metastasis. J Natl Cancer Inst 84:1588–1592.

Fidler IJ, Kitadai Y, Takahashi Y, Ellis LM. 1996. Molecular determinants of human colon cancer metastasis. In: Tahara E, Sugimachi K, Dohara T, editors. Recent advances in gastroenterological carcinogenesis. Vol 1. Bologna: Monduzzi Editore. p 3–9.

Gumley TP, McKenzie IF, Sandrin HS. 1995. Tissue expression, structure and function of the murine Ly-6 family of molecules. Immunol Cell Biol 73:277–296.

Hakomori S-I. 1996. Tumor malignancy defined by aberrant glycosylation and sphingo(glyco)lipid metabolism. Cancer Res 56:5309–5318.

Halachmi E, Witz IP. 1989. Differential tumorigenicity of 3T3 cells transformed in-vitro with Polyoma virus and the in-vivo selection for high tumorigenicity. Cancer Res 49:2383–2389.

Katz B-Z, Eshel R, Sagi-Assif O, Witz IP. 1994. An association between high Ly-6A/E expression on tumor cells and a highly malignant phenotype. Int J Cancer 59:684–691.

Katz B-Z, Ishai-Michaeli R, Zusman T, Vlodavsky I, Witz IP. 1994–95. Lung colonization by and heparanase activity in in-vitro transformed 3T3 cells rendered highly tumorigenic by an in-vivo passage. Invasion Metast 14: 276–289.

Kibbey MC, Grant DS, Kleinman HK. 1992. Role of the SIKVAV site of laminin in promotion of angiogenesis and tumor growth: an in vivo Matrigel model. J Natl Cancer Inst 84:1633–1638.

Kimura Y, Furuhata T, Urano T, Hirata K, Nakamura Y, Tokino T. 1997. Genomic structure and chromosomal localization of GML (GPI-anchored molecule-like protein), a gene induced by p53. Genomics 41:477–480.

Kinzler KW, Vogelstein B. 1996. Lessons from hereditary colorectal cancer. Cell 87:159–170.

Kong RKM, Barrios A, Knapp W, Macher BA. 1993. Fucosylated glycosphingolipids of human myeloid cells. Arch Biochem Biophys 300:677–683.

Leek RD, Lewis CE, Whitehouse R, Greenall M, Clarke J, Harris AL. 1996. Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. Cancer Res 56:4625–4629.

Liang P, Pardee AB. 1992. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science 257:967–971.

Mao M, Yu M, Tong J-H, Ye J, Zhu J, Huang Q-H, Fu G, Yu L, Zhao S-Y, Waxman S, Lanotte M, Wang Z-Y, Tan J-Z, Chan S-J, Chen Z. 1996. *RIG-E*, a human homolog of the murine Ly-6 family, is induced by retinoic acid during the differentiation of acute promyelocytic leukemia cell. Proc Natl Acad Sci USA 93:5910–5914.

Neumark E, Anavi R, Witz IP, Ben-Baruch A. 1999. MCP-1 expression as a potential contributor to the high malignancy phenotype of murine mammary adenocarcinoma cells. Immunology Letters (in press).

Nicolson GL. 1991. Quantitative variations in gene expression: possible role in cellular diversification and tumor progression. J Cell Biochem, 46:277–283.

Quak JJ, Balm AJM, Van Dongen GAMS, Brakkee JGP, Scheper RJ, Snow GB, Meijer CJLM. 1990. A22-kd surface antigen detected by monoclonal antibody E48 is exclusively expressed in stratified squamous and transitional epithelia. Am J Pathol 136:191–197.

Radinsky R, Fidler IJ. 1992. Regulation of tumor cell growth at organ-specific metastasis. In Vivo 6:325–332.

Ramani P, Balkwill F. 1987. Enhanced metastases of a mouse carcinoma after in-vitro treatment with murine interferon gamma. Int J Cancer 40:830–834.

Ran M, Katz B, Kimchi N, Halachmi E, Teillaud J-L, Even J, Berko Y, Atlas E, Fridman WH, Witz IP. 1991. The in-vivo acquisition of FcγRII expression on polyomas virus transformed cells derived from tumors of long latency. Cancer Res 51:612–618.

Rock KL, Reiser H, Bamezi A, McGrew J, Benacerraf B. 1989. The Ly-6 locus: a multigene family encoding phosphatidylinositol-anchored membrane proteins concerned with T cell activation. Immunol Rev 111:195–224.

Sager R. 1997. Expression genetics in cancer: Shifting the focus from DNA to RNA. Proc Natl Acad Sci USA 94:952–955.

Sagi-Assif O, Treister A, Katz B-Z, Anavi R, Eskenasy M, Witz IP. 1996. TNFα and anti Fas antibodies regulate Ly-6E.1 expression by tumor cells. A possible link between angiogenesis and Ly-6E.1. Immunol Lett 54:207–213.

Shevach EM, Korty PE. 1989. Ly-6: a multigene family in search of function. Immunol Today 10:195–200.

Springer TA, Lasky LA. 1991. Sticky sugars for selectins. Nature 349:196–197.

Sun J, Thurin J, Cooper HS, Wang P, Mackiewicz M, Steplewski Z, Blazczyk-Thurin M. 1995. Elevated expression of H type GDP-L-fucose: β-D-Galactosidase α-2-L-fucosyltransferase is associated with human colon adenocarcinoma progression. Proc Natl Acad Sci USA 92:7524–7528.

Treister A, Sagi-Assif O, Meer M, Smorodinsky NI, Anavi R, Golan I, Meshel T, Kahana O, Eshel R, Katz B-Z, Shevach E, Witz IP. 1998. Expression of Ly-6, a marker for highly malignant murine tumor cells, is regulated by growth conditions and stress. Int J Cancer 77:306–313.

Tonetti M, Sturla L, Bisso A, Benatti U, De Flora A. 1996. Synthesis of GDP-L-fucose by the human FX protein. J Biol Chem 271:27274–27279.

Walz G, Aruffo A, Kolanus W, Bevilacqua M, Seed B. 1990. Recognition by ELAM-1 of the sialyl-LeX determinant on myeloid and tumor cells. Science 250:1132–1135.

Witz IP. 1977. Tumor-bound immunoglobulins. In-situ expression of tumor immunity. Adv Cancer Res 25:95–148.

Witz IP, Sagi-Assif O, Ran M. 1996. The shaping of the malignancy phenotype—an interplay between cellular characteristics and microenvironmental factors. In: Yefenof E, Scheuermann RH, editors. Premalignancy and tumor dormancy. Austin, TX: Medical Intelligence Unit, R.G. Landes Co. p 147–156.

Yamamura K, Kibbey MC, Jun SH, Kleinman HK. 1993. Effect of Matrigel and laminin peptide YIGSR on tumor growth and metastasis. Semin Cancer Biol 4:259–265.

Zhang L, Zhou W, Velculescu VE, Kern SE, Hruban RH, Hamilton SR, Vogelstein B, Kinzler KW. 1997. Gene expression profiles in normal and cancer cells. Science 276:1268–1272.

Zusman T, Gohar O, Eliassi I, Avivi Y, Lisansky E, Sautes C, Even J, Bonnerot C, Fridman WH, Witz IP, Ran M. 1996a. The murine Fc-gamma (Fcγ) receptor type II B1 is a tumorigenicity-enhancing factor in polyoma-virus-transformed 3T3 cells. Int J Cancer 65:221–229.

Zusman T, Lisansky E, Arons E, Anavi R, Bonnerot C, Sautes C, Fridman WH, Witz IP, Ran M. 1996b. The contribution of the intracellular domain of the murine Fc-gamma receptor type II B1 to its tumor enhancing potential. Int J Cancer 67:1–9.