Effect of transforming growth factor-β1, insulin-like growth factor-I, and hepatocyte growth factor on proteoglycan production and regulation in canine melanoma cell lines

Montserrat Serra, DVM; Josep Pastor, DVM, PhD; Clelia Domenzain, MS; Anna Bassols, PhD

The extracellular matrix (ECM) plays an important role in the regulation of complex processes such as cell proliferation, adhesion, and migration. Metastasis of tumors involves a series of events, including adhesion and migration of tumor cells. Many steps in metastasis of melanomas involve cell-cell or cell-matrix interactions. Therefore, surface molecules that mediate these processes play an important role in regulating dissemination of melanomas, and their degree of expression may alter during the course of tumor progression. Proteoglycans (PG) are major components of the ECM, and they play a fundamental role in maintenance of the structure and function of the ECM.

Proteoglycans are glycoproteins bearing sulfated polysaccharide chains with repeating disaccharide units termed glycosaminoglycans (GAG). They are mainly found in the cell surface, within basement membranes, and in the ECM. Composition of the GAG chain differs among PG. This differing composition is the basis for classification of GAG into 4 large families: heparan sulfate and heparin, chondroitin sulfate and dermatan sulfate, keratan sulfate, and hyaluronic acid. The basic structures of these GAG have been summarized elsewhere. Heparan sulfate PG and chondroitin sulfate PG (CSPG) have been implicated in the control of cell proliferation, differentiation, and modulation of cell adhesion. Altered PG have been described in tumors and transformed cells and are believed to contribute to the abnormal assembly of the ECM in those cells.

Several CSPG have been identified in melanoma cells, and it has been proposed that they are essential regulators of cell adhesion, proliferation, and migration. Our research group has described finding versican, a PG with a large molecular weight, in human melanoma cell lines and in malignant melanomas in vivo and has related the production of versican by melanoma cells to a change in the biological properties that would decrease adherence of these cells to a tumor and increase their ability to metastasize. Versican belongs to the family of large aggregating PG that also includes aggrecan, the large cartilage-derived PG, and 2 smaller PG expressed in nervous tissues (ie, neurocan and brevican). These PG have 3 structural domains. The N-terminal domain binds hyaluronic acid, the central domain carries the GAG side chains, and the C-terminal region may interact with simple carbohydrates and GAG and probably with other matrix proteins. The central domain of versican consists of 2 large subdomains, designated GAG-α and GAG-β, that are encoded by 2 alternatively spliced exons. There are 4 possible spliced variants of mammalian versican. The

Objective—To identify extracellular proteoglycans produced by canine melanoma cell lines and analyze the effect of transforming growth factor-β1 (TGF-β1), insulin-like growth factor-I (IGF-I), and hepatocyte growth factor (HGF) on these proteoglycans.

Sample Population—3 canine melanoma cell lines (ie, CML-1, CML-6M, and CML-10c2).

Procedure—Extracellular proteoglycans were analyzed by use of metabolic labeling and western immunoblot analysis. The effect of TGF-β1 on cell proliferation was determined by incorporation of 5-bromo-2'-deoxyuridine.

Results—The CML-1 and CML-6M melanoma cell lines produced 2 main extracellular proteoglycans. One of them was identified as versican, a proteoglycan found in undifferentiated human melanoma cell lines. The CML-10c2 cells produced a small amount of extracellular proteoglycans. Addition of TGF-β1 (1.25 to 6.25 ng/ml) increased the release of sulfated proteoglycans into the medium. The TGF-β1 had mainly a posttranslational effect, because it increased the molecular mass of the sulfated bands. Addition of IGF-I (50 ng/ml) slightly increased production of proteoglycans in the CML-6M cell line, whereas HGF (50 ng/ml) did not have any effect on proteoglycan production.

Conclusions and Clinical Relevance—The proteoglycan content and response to TGF-β1 treatment for CML-1 and CML-6M canine melanoma cell lines are similar to that for undifferentiated human melanoma cell lines. In contrast, CML-10c2 cells produced a low amount of proteoglycans with high molecular weight. Because these extracellular proteoglycans are involved in the control of cell adhesion, proliferation, and migration, they may play an important role in the progression of melanomas in dogs. (Am J Vet Res 2002;63:1151–1158)
largest variant contains GAG-α and GAG-β and is designated V0; the other variants are V1 (contains only GAG-β), V2 (contains only GAG-α), and V3 (lacks both GAG subdomains). Other CSPG in human melanoma cells are melanoma-specific PG (ie, mel-CSPG) and the hyaluronate receptor (ie, CD44). Hormones and growth factors can modulate the synthesis of PG. Among these agents, transforming growth factor-β (TGF-β) is 1 of the most powerful. Transforming growth factor-β is able to induce PG as well as other components of the ECM such as fibronectin, collagens, and integrins. Furthermore, TGF-β affects the PG components of the ECM in diverse ways, depending on the target cell and the type of PG. It is able to induce the production of biglycan, versican, and perlecan at the transcriptional level in some cell types, but it does not affect or may down-regulate the synthesis of the decorin core protein. In addition, TGF-β can have an effect at the posttranslational level, increasing the length or total mass of the chondroitin sulfate chains in biglycan, decorin, versican, and syndecan, and CD44; however, TGF-β does not affect heparan sulfate chains.

In human melanoma cells, TGF-β1 is able to induce the synthesis of extracellular PG, including mel-CSPG, at the posttranslational level as a result of increases in the size of the GAG chains. The effect of TGF-β1 is related to the degree of cell differentiation, because the effect on PG release and changes in disaccharide composition and sulfation of GAG chains are more pronounced in undifferentiated cell lines, whereas it does not have an effect in differentiated cells.

The objective of the study reported here was to analyze the extracellular PG produced by 3 canine melanoma cell lines through comparison with extracellular PG produced by human melanoma cell lines. We also intended to study the effects of TGF-β1, insulin-like growth factor I (IGF-I), and hepatocyte growth factor (HGF) on production of these PG.

Materials and Methods

Cell cultures—Several cell lines were used in the study. Canine melanoma cell lines CML-1, CML-6M, and CML-10c2 were originally derived from melanoma tumors of dogs. Human U251 astrocytoma cells and human melanoma SK-mel-131 (cl 1.36-1-5) and SK-mel-23 cell lines were originally derived from melanoma tumors of humans.

Cells were grown in a humidified atmosphere at 37 C with 5% CO₂ in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 U of penicillin/ml, and 100 μg of streptomycin/ml. Subconfluent cultures were grown in serum-free medium for 4 hours. Cells then were labeled by incubation for 24 hours with carrier-free [35S]sulfate (100 μCi/ml) or [3H]methionine (25 μCi/ml). Medium was removed, and a cocktail of protease inhibitors was added (10 mM EDTA, 5 mM benzamidine, 5 mM N-ethylmaleimide, and 1 mM phenylmethylsulfonyl fluoride). The product was analyzed by use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a 6% or a 3 to 10% gradient polyacrylamide gel, which was followed by fluorography.

In the case of treatment with growth factors, subconfluent cultures were grown in serum-free medium for 4 hours, and cells then were labeled as described previously, using concentrations of TGF-β1 (range, 1.25 to 6.25 ng/ml), IGF-I (50 ng/ml), or HGF (50 ng/ml) for 24 hours. The 3 factors were of human origin. Radioactivity in the polyacrylamide gels was analyzed by use of a phosphor imaging system, and densitometry was performed by use of image analysis software. Statistical analysis was performed by use of an ANOVA, using a statistical software program.

Cell proliferation assay—Cells were plated in 96-well tissue culture plates and grown for 48 hours to 50% confluence in RPMI 1640 medium supplemented with 10% fetal calf serum. Cells then were incubated for 4 hours in serum-free medium. Medium was changed again, TGF-β1 at various concentrations was added to the wells, and the plates were incubated for another 24 hours. Cell proliferation was measured by incorporation of 5-bromo-2′ deoxyuridine, using a commercially available kit in accordance with the manufacturer’s instructions. The response to TGF-β1 was compared with that of the Mv1Lu mink lung epithelial cell line; growth of the mink lung epithelial cell line is strongly inhibited by TGF-β1.

Enzymatic digestions—Enzymatic digestions were performed at 37 C for 16 hours by use of chondroitinase ABC (50 mU/ml in a solution of 33 mM sodium acetate and 33 mM Tris-HCl, pH 8.0) or heparinase (10 mU/ml in a solution of 10 mM calcium acetate and 100 mM sodium acetate; pH 7.0). Incubations were terminated by boiling the samples for 5 minutes.

Western blot analysis—Aliquots of conditioned media were digested with chondroitinase ABC as described previously and analyzed in a 3 to 10% gel, using reducing conditions. After electrophoresis, proteins were transferred onto polyvinylidene difluoride membranes. Membranes were placed in a blocking solution consisting of 5% skim milk in Tris buffer saline (TBS)-0.05% Tween 20 and incubated for 1 hour at 25 C. After blocking, membranes were incubated with a polyclonal antibody against versican raised in our laboratory and with monoclonal antibody B5 raised against human mel-CSPG in 5% skim milk in TBS for 16 hours, washed, and developed by use of chemiluminescence. The antibody against versican recognizes the protein core of the molecule, but it is unable to detect the entire PG. The monoclonal antibody B5 against mel-CSPG also recognizes an epitope in the protein core of the mel-CSPG molecule.

Results

Molecular analysis of extracellular PG from canine melanoma cell lines—Newly synthesized PG were metabolically labeled with [35S]sulfate and analyzed by use of gel electrophoresis. The CML-1 and CML-6M cells produced 2 high-molecular-weight PG as the main sulfated extracellular molecules; 1 of them remained at the entrance of the gel, and the other migrated to a location above the 200-kd marker (Fig 1). A third band of lesser intensity and lower molecular mass was also visible for CML-1 and CML-6M cells. Sulfated bands for the CML-1 cells appeared to have a higher molecular mass than corresponding bands for the CML-6M cells. The 3 bands were characterized as CSPG on the basis of results of enzyme digestion, because they were completely degraded after treatment with chondroitinase ABC, whereas they were not affected by heparinase. The CML-10c2 cells yielded extremely small amounts of sulfated molecules into the extracellular medium.
Western blot analysis of PG produced by canine melanoma cell lines revealed 2 bands that were recognized by the antiversican antibody (Fig 2). Metabolic labeling experiments revealed that CML-1 and CML-6M cells produced versican, most probably the V0 and V1 isoforms. In contrast, CML-10c2 did not produce any versican isoform. In this aspect, it was similar to the differentiated human SK-mel-23 cell line. A third band recognized by the antibody was evident in canine melanoma cell lines but not in human cells. Although it was not identified, its molecular weight did not correspond to any versican isofrom.

We also tested for melanoma-specific PG (ie, mel-CSPG) by use of western blot analysis with monoclonal antibody B3 raised against human mel-CSPG. We did not identify any bands, probably because of the lack of cross-reactivity of the antibody against the canine molecule.

Effect of TGF-β, IGF-1, and HGF on PG production by canine melanoma cell lines—Addition of TGF-β to culture medium increased the release of PG into the extracellular medium for the CML-1 and CML-6M cells (Fig 3). In this case, the stacking gel was used to characterize versican. Use of TGF-β had a double effect, because it increased the intensity of the sulfated bands and also decreased the mobility of the bands through the gel, indicating that the PG molecules had an increase in molecular mass. This increase in molecular mass was more noticeable in the lower molecular weight band, but this possibly was attributable to the fact that it is easier to see a change in this area of the gel. The effect was evident even at 1.25 ng/ml (ie, 50 pM), the lowest TGF-β concentration assayed. The effect was maximal at 2.5 ng/ml and slightly decreased at 6.25 ng/ml. The increase in intensity produced by 2.5 ng of TGF-β/ml was calculated by use of densitometry as 1.9-fold for versican and 1.8-fold for the low-molecular-weight band for the CML-1 cells and 4.5-fold for versican and 2.9-fold for the low-molecular-weight band for the CML-6M cells.

Relative intensities of both bands in the 2 cell lines differed significantly (P = 0.006). On the contrary, there was not a significant effect of TGF-β on PG production by the CML-10c2 cells.

To ascertain whether the increase in band intensity provoked by TGF-β was attributable to an increase in the protein core, CML-1 cells were metabolically labeled with [35S]methionine and treated with TGF-β. The conditioned media were used without (control) or with chondroitinase ABC (ChABC) or heparitinase (Hase). Medium corresponding to the same number of cells was analyzed by use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a 3 to 10% gel followed by fluorography. Bands of high-molecular-weight PG were evident for CML-1 (arrows) and CML-6M (arrowheads) cells. The experiment was performed 4 times with similar results.
Conditioned media were digested with chondroitinase ABC to degrade chondroitin sulfate chains, and the protein core from versican (V0 and V1 isoforms) was determined after SDS-PAGE and fluorography (Fig 4). There was not a significant difference in the amount of protein core after treatment with TGF-β1, indicating that the increase in intensity was probably the result of an increase in the GAG chains, at least in the case of versican. This fact also indicated that the action of TGF-β1 was not attributable to a general effect on protein synthesis.

The 3 canine melanoma cell lines were treated with IGF-I and HGF and simultaneously labeled with [35S]sulfate. The IGF-I had a slight but significant stimulatory effect on the 3 cell lines (CML-1, 1.03-fold for versican and 1.32-fold for the low-molecular-weight

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**Figure 4**—Representative fluorogram revealing the effect of TGF-β1 on the core protein of versican from CML-1 and U-251 cells. Conditioned media from control and TGF-β1-treated CML1 cells labeled with [35S]methionine were digested by use of chondroitinase ABC and analyzed by SDS-PAGE in a 3 to 10% gel followed by fluorography. Notice the 2 bands corresponding to the V0 and V1 isoforms of versican (arrows). Cells of a human astrocytoma cell line (U251) were treated in an identical manner and served as an internal control sample. The experiment was performed 3 times with similar results.

**Figure 5**—Representative fluorogram revealing the effect of IGF-I on extracellular PG produced by canine melanoma cell lines. Subconfluent CML1, CML6M, and CML10c2 cells were labeled with [35S]sulfate with (I) or without (C) 50 ng of IGF-I/ml. The conditioned media corresponding to the same number of cells was analyzed by use of SDS-PAGE in a 3 to 10% gel followed by fluorography. The experiment was performed 3 times with similar results.

**Figure 6**—Representative fluorogram revealing the effect of hepatocyte growth factor (HGF) on extracellular PG produced by canine melanoma cell lines. Subconfluent CML1, CML6M, and CML10c2 cells were labeled with [35S]sulfate with (H) or without (C) 50 ng of HGF/ml. The conditioned media corresponding to the same number of cells was analyzed by use of SDS-PAGE in a 3 to 10% gel followed by fluorography. The experiment was performed 3 times with similar results.

**Figure 7**—Representative graph depicting the effect of TGF-β1 on the proliferation of canine melanoma cell lines. Cells of CML1, CML6M, and CML10c2 and mink lung epithelial (Mv1Lu) cells were seeded in 96-well tissue culture plates containing serum-free medium. Various concentrations of TGF-β1 were added, and plates were incubated for 24 hours. Cell proliferation was evaluated by determining the incorporation of 5-bromo-2’-deoxyuridine. Values reported are mean ± SEM of triplicate determinations. The experiment was performed 4 times with similar results. OD450 nm = Optical density measured at a wavelength of 450 nm.
versican is also able to decrease cell adhesion and increase cell migration. Versican has been identified in human melanoma cell lines and in malignant melanomas, and it is a key regulator of cell adhesion, proliferation, and cell behavior, and it has been proposed that they are essential regulators of cell adhesion, proliferation, and migration. Versican has been identified in human melanoma cell lines and in Mv1Lu cells, which were used as a control population. The TGF-β1 treatment for the same conditions (data not shown), which indicated that TGF-β1 did not affect proliferation or apoptosis in these canine melanoma cell lines.

**Effect of TGF-β1 on proliferation of canine melanoma cell lines**—The effect of TGF-β1 on cell proliferation was analyzed in all 3 canine melanoma cell lines and in Mv1Lu cells, which were used as a control population. The TGF-β1 potently inhibited the growth of Mv1Lu cells, as expected, whereas none of the canine melanoma cell lines was significantly affected by TGF-β1 treatment (Fig 7). Total number of cells was not altered after TGF-β1 treatment for the same conditions (data not shown), which indicated that TGF-β1 did not affect proliferation or apoptosis in these canine melanoma cell lines.

**Discussion**

Several CSPG have been involved in melanoma cell behavior, and it has been proposed that they are essential regulators of cell adhesion, proliferation, and migration. Versican has been identified in human melanoma cell lines and in malignant melanomas, and it is a key regulator of cell adhesion and increase cell proliferation in human melanomas. Versican is also produced by a number of malignant conditions in humans such as histiocytes, breast cancer, prostate cancer, and some nonepithelial neoplasms. The results reported here indicated that versican is also produced by canine melanoma cell lines.

In the study reported here, we found that some canine melanoma cell lines produced versican (CML-1 and CML-6M), whereas other cell lines did not produce this PG (CML-10c2). In the case of human cells, the expression of versican has a close relationship to the degree of cell differentiation, because it is expressed in undifferentiated cell lines but is not detected in differentiated cell lines. It is tempting to speculate that the CML-10c2 cell line represents a more differentiated phenotype of melanoma cells. Nevertheless, we do not have sufficient data to confirm this assertion, and a more detailed molecular characterization of these cell lines must be performed. The versican isoforms produced by the CML-1 and CML-6M cells were the V0 and V1 isoforms, similar to the isoforms produced by human melanoma cell lines. Several types of brain tumors in humans. We did not detect indications of a possible differential role of versican isoforms, although it is feasible that the difference in size of the GAG domain (V0 > V1 > V2 and totally lacking in V3) could modulate the biological and binding properties of versican. Such modulation has been suggested for the nervous system, where versican is able to inhibit axonal growth.

Versican probably has a role in biological functions of melanoma cells. Purified versican is able to increase the proliferation rate of undifferentiated human melanoma cells. Other in vitro results also support a role for versican in cell proliferation, because cells transfected with a minigene containing the terminal domains of versican grow more rapidly. Furthermore, versican is up-regulated in keratinocytes grown in proliferation-promoting conditions, whereas versican is down-regulated in culture conditions promoting keratinocyte differentiation. In vivo, versican is abundant throughout the entire dermis early during the fetal period in humans, a period in which proliferation occurs at a high rate; however, versican disappears progressively from the lower half of the dermis during the fetal period.

In addition to increasing cell proliferation, versican has an antiadhesive effect, similar to that of many CSPG, probably as a result of the chondroitin sulfate chains. We have documented that versican is a counteradhesive substrate for human melanoma cells grown in various ECM components such as fibronectin and collagen 1. Versican is excluded from focal contacts of cultured fibroblasts, suggesting that it could inhibit cell adhesion by repressing formation of focal contacts. The detection of versican correlates with the production of hyaluronate in melanomas. Because versican and hyaluronate interact through the N-terminal domain of versican, they could create versican-hyaluronate-rich matrices that would form an expanded and hydrophilic environment deforming the normally compact architecture of ECM and facilitating cell movement. These molecular changes in the ECM could then contribute to the higher mobility of undifferentiated melanoma cells and the increased ability to migrate and develop metastatic lesions.

The other extracellular PG identified in melanomas of humans is melanoma-specific PG (ie, mel-CSPG). This membrane CSPG consists of a 250-kd glycoprotein core with several GAG chains that make up a complete PG of 400 to 1,000 kd. It is released into the medium as a result of proteolytic cleavage of membrane forms. Furthermore, mel-CSPG supposedly has several important roles in regulating melanoma cell adhesion, proliferation, and migration. The CML-1 and CML-6M cells produced versican and another CSPG of high molecular mass (approx 400 kd), which possibly corresponds to the canine version of mel-CSPG. Unfortunately, the antibody against human mel-CSPG (ie, monoclonal antibody B5) did not recognize any band for the canine melanoma cells, probably indicating a lack of cross-reactivity between the molecules of these 2 species.

Although CML-1 and CML-6M cells produced a similar set of extracellular PG, a general difference was observed in the molecular mass of these molecules, because all of them had a higher molecular mass for CML-1 than for CML-6M. This probably was attributable to a difference in the length or number of GAG chains, at least in the case of versican, because the protein core is the same for both cell lines. The CML-1 and CML-6M cells also appeared to have differing relative amounts of PG, and these differences could have a role in the particular behavior of these melanoma cell lines.

Melanoma cells produce a large number of growth factors and cytokines that enable them to grow in the particular behavior of these melanoma cell lines.
autonomously and confer competence to enable them to metastasize.\textsuperscript{31} On the other hand, the structure and composition of PG, as well as other components of the ECM, are under the control of growth and differentiation factors. Transforming growth-factor-\(\beta\) exerts a negative influence on cell proliferation of normal melanocytes, but advanced-stage melanoma cells become resistant to the antiproliferative effect of TGF-\(\beta\).\textsuperscript{27,28} The canine melanoma cell lines examined in the study reported here were not susceptible to the growth-inhibitory properties of TGF-\(\beta\), although they were still able to respond to TGF-\(\beta\) treatment. This fact indicates that these canine melanoma cells do express functional TGF-\(\beta\) receptors, similar to the situation described for human cells.\textsuperscript{31,32} It is recognized that unresponsiveness to TGF-\(\beta\) may be heterogeneous in terms of its biological effects and that several mechanisms may cause this phenomenon. For example, lack of expression of the gene coding for the receptor (mostly the TGF\(\beta\)RII gene) as a result of genetic or epigenetic events can be the cause of unresponsiveness, although many times tumor cells appear to express functional receptors.\textsuperscript{33} This is the case for canine melanoma cells, because they still respond to TGF-\(\beta\) by producing increasing amounts of PG. A mutation in a component of the signal transduction machinery or in the transcriptional machinery of the cells could be the cause of this differential effect of TGF-\(\beta\). In this sense, lack of p15 or overexpression of c-Myc renders the cells resistant to the growth inhibitory activity of TGF-\(\beta\).\textsuperscript{34} Clearly, additional studies should be conducted to clarify this issue in canine melanoma cells.

In addition to its effects on cell proliferation, TGF-\(\beta\) is unique because of its potency as an inducer of PG and other ECM molecules. This potent effect has been associated with the ability of TGF-\(\beta\) to influence cell growth and differentiation.\textsuperscript{35} In the case of CML-1 and CML-6M cells, TGF-\(\beta\) induces the production of extracellular CSPG, versican, and, probably, mel-CSPG, similar to its effects in undifferentiated human melanoma cell lines.\textsuperscript{27,28} This induction is observed as an increase in the intensity of the sulfated band as well as in the molecular mass of the molecule, and it is probably the result of an increase in the length or number of GAG chains, at least for CML-1 cells, because it does not affect the amount of the protein core after metabolic labeling with [\(35\)S]methionine. Increased amounts of secreted PG are not attributable to an increase in cell number, because TGF-\(\beta\) does not have any effect on proliferation of canine melanoma cells. This result is not unexpected, because the effects of TGF-\(\beta\) primarily vary with the target cell and type of PG. Thus, it could affect posttranslational processing of GAG chains by increasing the length or varying the disaccharide composition and degree of sulfation of the GAG chains, or it could affect transcription of the core protein.\textsuperscript{20,26} In addition to TGF-\(\beta\), we analyzed the effects of IGF-I and HGF on PG production. Both growth factors are active on melanocyte or melanoma cells. Thus, IGF-I is an essential mitogen for normal melanocytes, nevus cells, and early primary melanoma cells in vitro, and it is chemotactic for melanoma cells.\textsuperscript{35,37} On the other hand, HGF is a mitogen for human melanocytes and has been implicated as an important factor for the development and dissemination of melanomas.\textsuperscript{38,39} It has been reported\textsuperscript{40-42} that IGF-I is able to induce PG synthesis in several cell types, but it has only a slight stimulatory effect on CML-6M cells. Hepatocyte growth factor is able to interact with heparan PG and dermatan sulfate PG, which serve as coreceptors, and this interaction is critical for HGF activity.\textsuperscript{43,44} Analysis of results of the study reported here indicated that HGF did not have any effect on PG production by canine melanoma cells.

Extracellular PG produced by canine melanoma cell lines such as CML-1 and CML-6M are versican and another CSPG similar to mel-CSPG, and this result is similar to that observed in undifferentiated human melanoma cell lines such as SK-melanoma-1-5 or SK-melanoma-3.\textsuperscript{44,45} Transforming growth factor-\(\beta\) increases PG production in the extracellular medium and increases the molecular mass of these macromolecules, similar to the situation in human cells. Furthermore, CML-10c2 had an extremely low amount of high-molecular-weight PG and did not respond to TGF-\(\beta\); in these aspects, it was more similar to the differentiated human melanoma cell line SK-melanoma-23. Analysis of these results indicated that canine cell lines could be a good cellular system to study the molecular characteristics of melanomas and the role of PG in the tumorigenic process. Furthermore, these findings could have important implications on the identification of malignant phenotypes in canine melanomas.

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