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RESEARCH ARTICLE

MT1-MMP expression level status dictates the in vitro action of lupeol on inflammatory biomarkers MMP-9 and COX-2 in medulloblastoma cells

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Abstract Local inflammation-induced extracellular matrix structural changes are a prerequisite to neoplastic invasion by pediatric intracranial tumors. Accordingly, increased expression of matrix metalloproteinases MMP-2 and MMP-9, two inflammation-induced matrix metalloproteinases (MMPs), may further aid the transformed cells either to infiltrate adjacent tissues or to enter the peripheral circulation. In the context of neuroinflammation, MMP-9 has been linked to processes such as blood–brain barrier opening and invasion of neural tissue by blood-derived immune cells. Given its reported anti-inflammatory and anticancer properties, we investigated the in vitro pharmacological effects of lupeol, a diet-derived triterpenoid, on MMP-9 and cyclooxygenase (COX)-2 expressions in a pediatric medulloblastoma DAOY cell line model. Lupeol was unable to inhibit the increased MMP-9 and COX-2 expression in phorbol 12-myristate 13-acetate (PMA)-treated cells, but was rather found to synergize with PMA to induce both biomarkers' expression. A contribution of the membrane type-1 (MT1)-MMP was also revealed, since lupeol/PMA treatments triggered proMMP-2 activation, and that MT1-MMP gene silencing reversed the combined effects of lupeol/PMA on both MMP-9 and COX-2. The mRNA stabilizing factor HuR was also found increased in the combined lupeol/PMA treatment, suggesting stabilization processes of the MMP-9 and COX-2 transcripts. We postulate that lupeol's anti-inflammatory properties may exert better pharmacological action within low MT1-MMP expressing tumors. Furthermore, these evidences add up to the new pleiotropic molecular mechanisms of action of MT1-MMP, and prompt for evaluating the future in vitro pharmacological properties of lupeol under pro-inflammatory experimental set-up.

Keywords Medulloblastoma · MMP-9 · MT1-MMP · NF-κB · COX-2 · Lupeol

Abbreviations

COX	Cyclooxygenase
IL	Interleukin
MMP	Matrix metalloproteinase
MT1-MMP	Membrane type-1 MMP
NF-κB	Nuclear factor kappa B
PMA	Phorbol 12-myristate 13-acetate
PGE	Prostaglandin E

Introduction

The strong association assumed between inflammation and tumor progression has raised interest in the anti-inflammatory and chemopreventive properties of dietary phytochemicals (Béliveau and Gingras 2007; Wang et al. 2011). Accordingly, lupeol, a phytosterol and triterpene widely found in edible fruits and vegetables has been ascribed pleiotropic pharmacological activities that include anti-inflammatory, antimicrobial, antiprotozoal, antiproliferative, anti-invasive,

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and antiangiogenic capacity (Moreau et al. 2002; Saleem 2009). Evidence has also been provided that lupeol modulates the expression or activity of several contributors to inflammation such as cytokines IL2, IL4, IL5, ILβ, proteases, α-glucosidase, cFLIP, Bcl-2 and NF-κB (Siddique and Saleem 2011). Given the complexity of carcinogenic processes and the fact that microenvironment dictates the acquisition of a tumor invasive and inflammatory phenotype, the wide spectrum of molecular mechanisms of action that characterize the pharmacological activity of lupeol may possibly be envisioned in the optimization and development of therapeutic modalities. The specific combined anti-inflammatory and chemopreventive activities of lupeol within pro-inflammatory in vitro conditions have however been poorly investigated.

Medulloblastoma is the most common and one of the most deadly malignant brain tumors of infancy (Packer 2007; Kanu et al. 2009). In addition to high tumor resistance to ionizing radiation and to chemotherapy, proximity of medulloblastomas to cerebrospinal fluid greatly enhances metastasis rate in the central nervous system (Bao et al. 2006; Mazloom et al. 2010). As part of our continuous effort to understand the molecular links between inflammation and tumorigenesis, we recently demonstrated that tumor microenvironment dictated the acquisition of an invasive phenotype in medulloblastoma cancer stem cells (Annabi et al. 2008). Such phenotype was in part linked to increased expression of MMP-9 and MT1-MMP, two MMPs involved in inflammation, angiogenesis, as well as cancer cell migration and metastasis (Chambers and Matrisian 1997; Amalinei et al. 2010). More importantly, a fundamental new role of MT1-MMP, besides its classical role in proMMP-2 activation, was later demonstrated in bioactive lysophospholipid signaling (Annabi et al. 2009), NF-κB-mediated cyclooxygenase (COX)-2 regulation (Han et al. 2001; Annabi et al. 2009b; Sina et al. 2010), and as a cell death sensor/effector (Belkaid et al. 2007; Currie et al. 2007; Proulx-Bonneau et al. 2011). Furthermore, while involvement of COX-2/PGE_2 pathway in the upregulation of MMP-9 was demonstrated in pancreatic cancer (Bu et al. 2011), specific pharmacological targeting of NF-κB was found to efficiently downregulate carcinogen-induced MMP-9 and COX-2 expression in brain endothelial cell as well as in medulloblastoma cell models (Annabi et al. 2010; Tahanian et al. 2011a).

Using a pediatric medulloblastoma-derived DAOY cell line model, we now assessed lupeol's multi-targeted capacity to temper pro-inflammatory stimulation, as induced upon phorbol 12-myristate 13-acetate-activated (PMA) treatment, on MMP-9 secretion and on COX-2 expression. MT1-MMP contribution was also evaluated in the transduction pathways modulated by the concerted action of lupeol/PMA.

Materials and methods

Materials

Sodium dodecylsulfate (SDS) and bovine serum albumin (BSA) were purchased from Sigma (Oakville, ON). Electrophoresis reagents were purchased from Bio-Rad (Mississauga, ON). The enhanced chemiluminescence (ECL) reagents were from Perkin Elmer (Waltham, MA). Micro bicinchoninic acid protein assay reagents were from Pierce (Rockford, IL). Lupeol was from Extrasynthese (Lyon, France) and reconstituted in ethanol as per the manufacturer's recommendation. The polyclonal antibody against MT1-MMP (AB815) was from Chemicon (Temecula, CA). The polyclonal antibody against IκBα C-terminus was purchased from Cell Signaling (Danvers, MA). The polyclonal antibody against HuR was from Santa Cruz Biotechnology (Santa Cruz, CA). The polyclonal antibody against COX-2 and monoclonal antibody against GAPDH were from Advanced Immunochemical Inc. (Long Beach, CA). Horseradish peroxidase-conjugated donkey anti-rabbit and anti-mouse IgG secondary antibodies were from Jackson ImmunoResearch Laboratories (West Grove, PA). All other reagents were from Sigma-Aldrich, Canada.

Cell culture

The human DAOY medulloblastoma cell line was purchased from American Type Culture Collection and was maintained in Eagle's Minimum Essential Medium containing 10 % (v/v) calf serum (HyClone Laboratories, Logan, UT), 2 mM glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin. Cells were incubated at 37 °C, with 95 % air and 5 % CO_2. Treatment was done in X-Vivo 15 from Lonza (Walkersville, MD) containing 20 ng/mL of basic fibroblast growth factor, 20 ng/mL of epidermal growth factor from Wisent (St-Bruno, QC), 20 ng/mL of leukemia-inhibitory factor from Sigma-Aldrich Canada and neural survival factor-1 also from Lonza.

cDNA synthesis and real-time quantitative RT-PCR

Total RNA was extracted from DAOY-derived cell monolayers using TriZol reagent (Life Technologies, Gaithersburg, MD). For cDNA synthesis, 2 µg of total RNA was reverse transcribed using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). cDNA was stored at −80 °C prior to PCR. Gene expression was quantified by real-time quantitative PCR using iQ SYBR Green Supermix (Bio-Rad, Hercules, CA). DNA amplification was carried out using an Icycler iQ5 (Bio-Rad), and product detection was performed by measuring binding of the fluorescent dye SYBR Green I to

double-stranded DNA. GAPDH primer sets were synthesized by Biocorp (Dollard-des-Ormeaux, QC) with the following sequences: forward CCATCA-CCATCTTC-CAGGAG and reverse CCTGCTTCACCACCTTCTTG. The following QuantiTect primer sets were provided by Qiagen (Valencia, CA): MMP-9 (QT00040040), COX-2 (QT00040586), MT1-MMP (QT00001533), HuR (QT0000 1533), and β-actin (QT01136772). The relative quantities of target gene mRNA compared against two internal controls, GAPDH and β-actin mRNA, were measured by following a ΔC_T method employing an amplification plot (fluorescence signal vs. cycle number). The difference (ΔC_T) between the mean values in the triplicate samples of target gene and those of GAPDH and β-actin mRNAs was calculated by iQ5 Optical System Software (v 2.0; Bio-Rad), and the relative quantified value was expressed as $2^{-\Delta C_T}$.

Transfection method and RNA interference

Cells were transiently transfected with 20 nM siRNA (Qiagen) against MT1-MMP (Hs_MMP14_6 FlexiTube siRNA, SI03648841) or scrambled sequences (AllStar Negative Control siRNA, 1027281) using Lipofectamine 2000 (Invitrogen, ON). Specific gene knockdown was evaluated by qRT-PCR as described above. Small interfering RNA and mismatch siRNA were synthesized by Qiagen (Valencia, CA) and annealed to form duplexes.

Gelatin zymography

Gelatin zymography was used to assess the extent of proMMP-9 activity as previously described (Belkaid et al. 2007). Briefly, an aliquot (20 µl) of the culture medium was subjected to SDS-PAGE in a gel containing 0.1 mg/ml gelatin. The gels were then incubated in 2.5 % Triton X-100 and rinsed in nanopure distilled H_2O. Gels were further incubated at 37 °C for 20 h in 20 mM NaCl, 5 mM $CaCl_2$, 0.02 % Brij-35, 50 mM Tris–HCl buffer, pH 7.6, then stained with 0.1 % Coomassie Brilliant blue R-250 and destained in 10 % acetic acid, 30 % methanol in H_2O. Gelatinolytic activity was detected as unstained bands on a blue background.

Immunoblotting procedures

Proteins from control and treated cells were separated by SDS–polyacrylamide gel electrophoresis (PAGE). After electrophoresis, proteins were electrotransferred to polyvinylidene difluoride membranes which were then blocked for 1 h at room temperature with 5 % non-fat dry milk in Tris-buffered saline (150 mM NaCl, 20 mM Tris–HCl, pH 7.5) containing 0.3 % Tween-20 (TBST). Membranes were further washed in TBST and incubated with the primary antibodies (1/1,000 dilution) in TBST containing 3 % bovine serum albumin, followed by a 1 h incubation with horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG (1/2,500 dilution) in TBST containing 5 % non-fat dry milk. Immunoreactive material was visualized by enhanced chemiluminescence (Amersham Biosciences, Baie d'Urfé, QC).

Statistical data analysis

Data are representative of three independent experiments. Statistical significance was assessed using Student's unpaired t test. Probability values of less than 0.05 were considered significant and an asterisk identifies such significance in the figures.

Results

COX-2 expression is increased upon lupeol/PMA synergistic actions through an NF-κB signaling pathway

We first wished to assess lupeol's actions within a pro-inflammatory in vitro environment (Annabi et al. 2010; Tahanian et al. 2011b). This was generated by the use of 1 µM PMA stimulation of serum-starved medulloblastoma-derived DAOY cells in the presence of various concentrations of lupeol for 18 h. As expected, we found that COX-2 expression was significantly increased upon PMA treatment (Fig. 1a, upper panel), and this required NF-κB signaling since IκB levels decreased (Fig. 1a, middle panel). Unexpectedly, when increasing concentrations of lupeol were concomitantly added COX-2 levels further synergistically increased inversely correlating with a parallel decrease in IκB expression (Fig. 1b). These results suggest new unreported effects of lupeol within pro-inflammatory culture conditions.

Concomitant increases in proMMP-9 secretion and proMMP-2 activation upon synergistic actions of lupeol/PMA

PMA is also well documented to induce MMPs secretion and/or activation processes (Ou et al. 2011; Tahanian et al. 2011b). We therefore harvested the conditioned media from above described conditions and used zymography to monitor both MMP-9 and MMP-2 gelatinolytic activities (Fig. 2a). We found that PMA alone significantly induced proMMP-9 secretion (Fig. 2b) and partially triggered latent

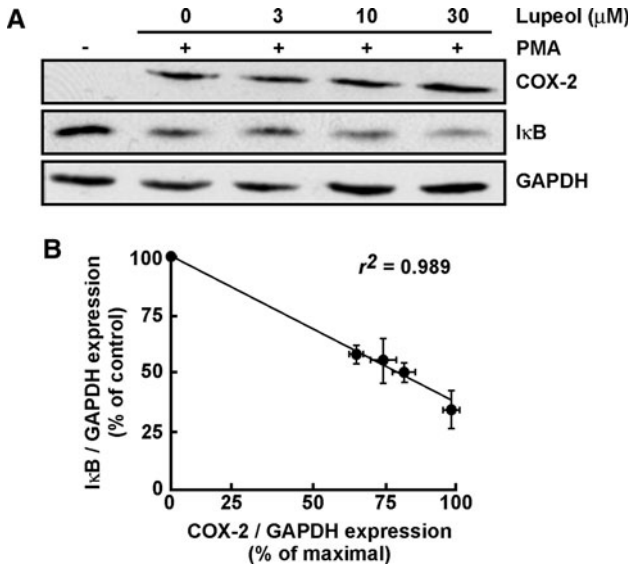

Fig. 1 COX-2 and MMP-9 expressions are increased upon synergistic actions of lupeol and PMA. DAOY cells were serum starved in the presence of various concentrations of lupeol in combination with vehicle (1 % ethanol) or 1 μM PMA for 18 h. **a** Lysates were isolated, electrophoresed via SDS-PAGE and immunodetection of COX-2, IκB and GAPDH proteins performed as described in the "Methods" section. **b** Quantification was performed by scanning densitometry of the respective autoradiograms from three independently performed experiments, data normalized on GAPDH, and correlation between IκB and COX-2 expression represented

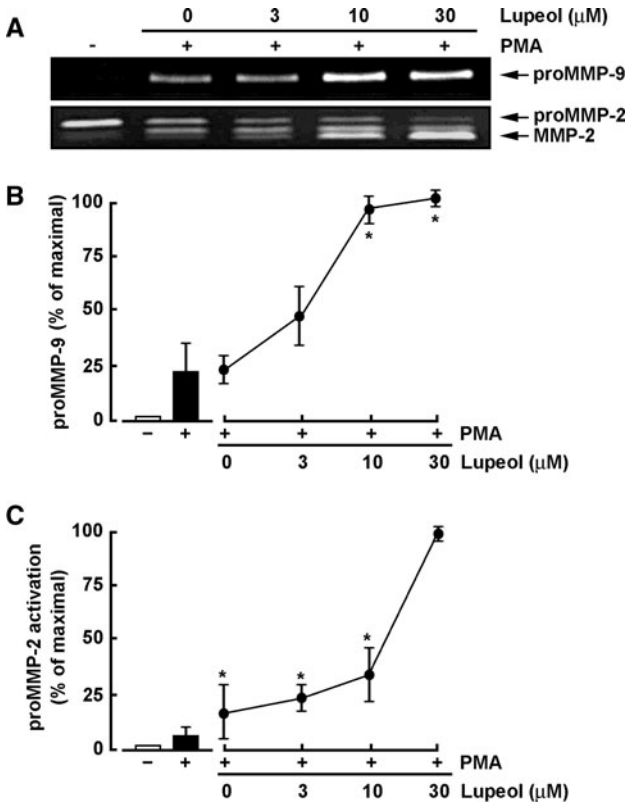

Fig. 2 Concomitant proMMP-9 secretion and proMMP-2 activation are increased upon synergistic actions of lupeol and PMA. DAOY cells were serum starved in the presence of various concentrations of lupeol in combination with vehicle or 1 μM PMA for 18 h. **a** Gelatin zymography was performed with the respective conditioned media to assess the extent of proMMP-9, proMMP-2, and MMP-2 activities. Scanning densitometry of the zymogram was performed to quantify the extent of proMMP-9 secretion (**b** probability values of less than 0.05 were considered significant and an *asterisk* identifies such significance with reference to PMA-treated cells), or the extent of proMMP-2 activation (**c** MMP-2/proMMP-2; probability values of less than 0.05 were considered significant and an *asterisk* identifies such significance with reference to PMA/30 μM lupeol-treated cells). Data are from four independently performed experiments

proMMP-2 processing into active MMP-2 (Fig. 2c). Again, addition of increasing concentrations of lupeol in the presence of PMA not only potentiated proMMP-9 secretion, but further triggered proMMP-2 activation. Collectively, this data add up to the pleiotropic targets of joint lupeol/PMA treatments, and further highlight potential new mechanisms possibly involving MT1-MMP in proMMP-2 activation and some mechanisms supporting proMMP-9 expression.

The synergistic lupeol/PMA effects require MT1-MMP

MT1-MMP signaling axis involvement in inflammation has been reported both directly (Sakamoto and Seiki 2009; Sina et al. 2010) and indirectly (Annabi et al. 2009b; Paquette et al. 2011). Here, in order to assess the joint contribution of lupeol/PMA treatment on COX-2 expression and on MMP-9 secretion, we used gene silencing strategies and transiently transfected DAOY cells with either scrambled (siScrambled) or MT1-MMP (siMT1-MMP) specific siRNA. Subsequent treatments were performed with lupeol, PMA, or lupeol/PMA as described above. MT1-MMP knockdown was confirmed at the protein level (Fig. 3a, upper panel). Consequently, the observed increases in COX-2 (Fig. 3b) and in MMP-9 (Fig. 3d) levels upon PMA or lupeol/PMA were all reduced without affecting GAPDH expression. Interestingly, significant reversal in the IκB expression was observed (Fig. 3c) and supports the MT1-MMP/NF-κB signaling axis as previously reported (Annabi et al. 2009b). In addition to the MT1-MMP contribution in the synergistic action of lupeol/PMA on COX-2 and MMP-9, we also found increased expression of HuR which was revealed upon lupeol/PMA treatment (Fig. 3e) supporting a potential contribution to the 3′-UTR stabilization of MMP-9 mRNA (Annabi et al. 2008, 2009c). Consequently, whether any transcriptional regulation of COX-2, MMP-9 and of HuR occurs upon lupeol/PMA was next investigated.

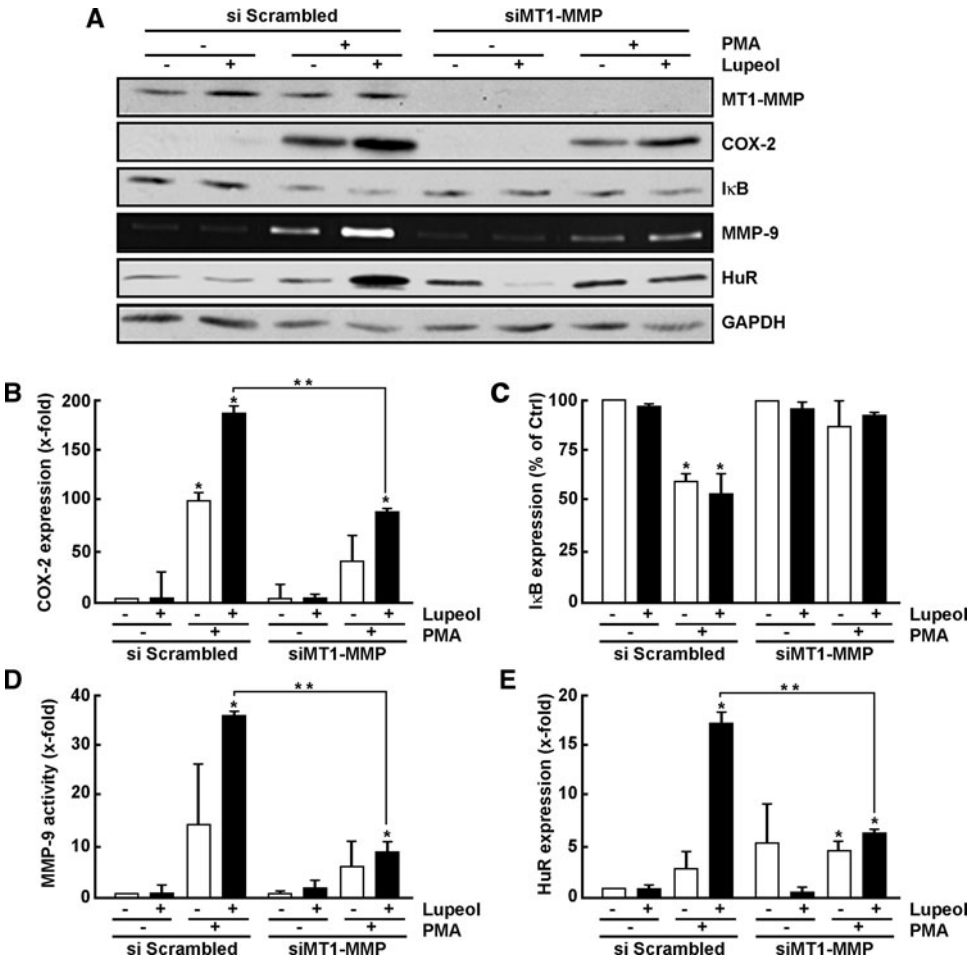

Fig. 3 The synergistic lupeol/PMA effects require MT1-MMP. DAOY cells were transfected with scrambled (siScrambled) siRNA sequences or with siMT1-MMP as described in the "Methods" section. The cells were then serum starved in the presence of 1 μM PMA and/or 30 μM lupeol for 18 h. **a** Lysates were isolated, electrophoresed via SDS-PAGE and immunodetection performed as described in the "Methods" section. Gelatin zymography also was used to assess the extent of proMMP-9 secretion in the conditioned media. Scanning densitometry was used to quantify the autoradiograms for **b** COX-2, **c** IκB, **d** proMMP-9, and **e** HuR respective expression. Densitometric values are mean of two independent experiments. Probability values of less than 0.05 were considered significant and an *asterisk* identifies such significance with reference to untreated cells. A *double asterisk* also indicates such significant probability between siScrmabled and siMT1-MMP cells treated with combined PMA/lupeol

MT1-MMP is required for the transcriptional regulation of COX-2 and MMP-9 upon synergistic actions of lupeol and PMA

Total RNA was extracted from the above siScrambled- or siMT1-MMP-transfected and treated DAOY cells. RT-PCR followed by qPCR enabled to assess transcriptional regulation of COX-2, MMP-9, and of HuR as described in the "Methods" section. Efficient MT1-MMP gene silencing was confirmed and small induction by PMA or lupeol/PMA observed (Fig. 4a) confirming the proMMP-2 activation previously observed by zymography. COX-2 gene expression was also induced by PMA and potentiated upon lupeol/PMA treatment (Fig. 4b). MT1-MMP gene silencing, however, did not significantly downregulate PMA or lupeol/PMA-induced COX-2 gene expression. In contrast, MMP-9 gene expression was also induced by PMA or lupeol/PMA, but MT1-MMP gene silencing resulted in decreased MMP-9 gene expression (Fig. 4c). When HuR gene expression levels were assessed, they remained relatively unchanged independent of the treatment (Fig. 4d). Collectively, we provide evidence for post-transcriptional events that regulate MT1-MMP-mediated regulation of HuR that would, in turn, regulate MMP-9 transcript stabilization leading to increased MMP-9 expression in lupeol/PMA-treated cells.

Discussion

Pharmacological effects of lupeol have been reported within inflammation-associated signaling conditions involved

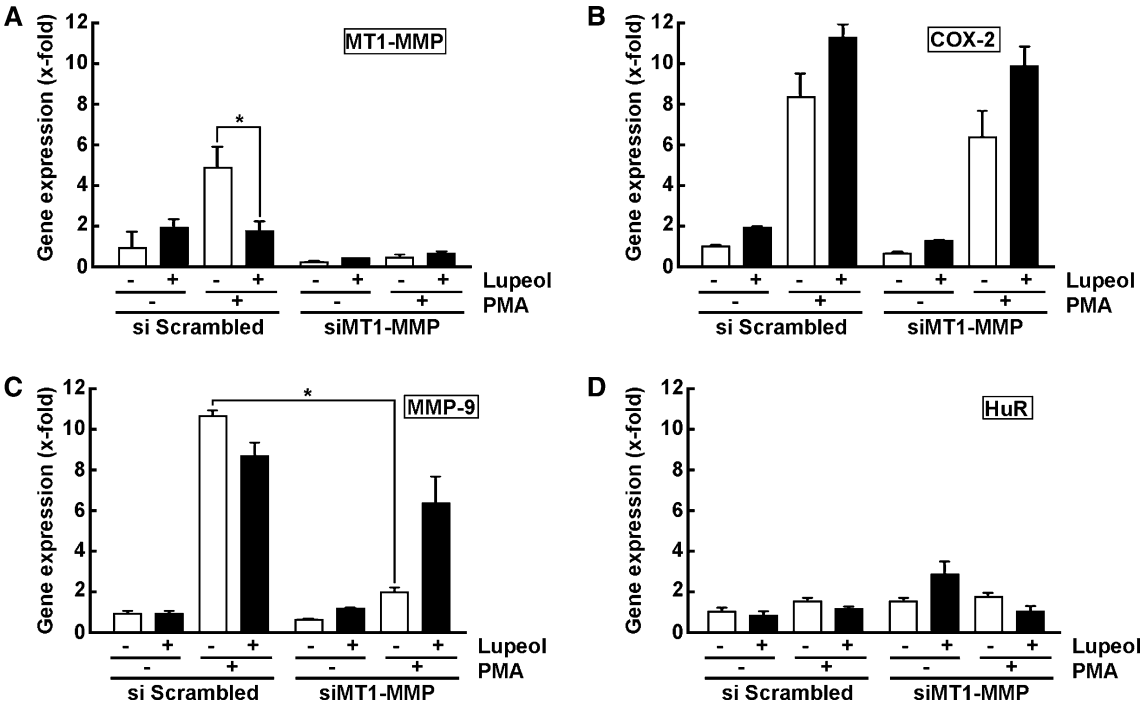

Fig. 4 MT1-MMP is required for the transcriptional regulation of COX-2 and MMP-9 upon synergistic actions of lupeol and PMA. DAOY cells were transfected with scrambled (siScrambled) siRNA sequences or with siMT1-MMP as described in the "Methods" section. The cells were then serum starved in the presence of 1 μM PMA and/or 30 μM lupeol for 18 h. Total RNA was extracted and transcribed into cDNA as described in the "Methods" section. qPCR was then performed in order to assess gene expression levels of MT1-MMP (**a**), COX-2 (**b**), MMP-9 (**c**), and HuR (**d**). Values are mean of two independent experiments assessed in triplicate. Probability values of less than 0.05 were considered significant and an *asterisk* identifies such significance

in wound healing, diabetes, cardiovascular disease, kidney disease, and arthritis (Geetha and Varalakshmi 1999; Sudhahar et al. 2006, 2007; Papi Reddy et al. 2009). Some of lupeol's anti-inflammatory properties arise, in fact, from its capacity to abrogate synthesis of pro-inflammatory mediators (Fernandez et al. 2001), and to interfere with several signaling pathways such as NF-κB, MAPK, Ras, Wnt and PI3 K/Akt (Saleem et al. 2004, 2005; Tarapore et al. 2010). Given that deregulation of these signaling pathways is often associated with carcinogenesis, various in vitro and in vivo models have further inferred lupeol to exhibit anti-cancer properties (Saleem 2009). While the sum of the multi-targeted effects of such a pharmacological agent is expected to impact on a variety of intracellular biomarkers expression, its intrinsic mechanism of action however remains poorly assessed within in vitro pro-inflammatory stimulation conditions.

In this study, we highlight an unexpected mechanism of action of lupeol in synergy with PMA-mediated inflammatory signaling such as one would expect to occur within a given tumor microenvironment. Synergistic induction of MMP-9 and of COX-2, two preeminent mediators of inflammation in pathological conditions, upon combined lupeol/PMA treatment is shown to require a crucial balance in MT1-MMP expression levels. MT1-MMP contribution to such signaling events was first inferred through its extracellular functions involved in proMMP-2 activation into MMP-2 (Fig. 2). Accordingly, lowering of MT1-MMP-mediated intracellular signaling through gene silencing strategies abrogated MMP-9 and COX-2 increases. We hypothesize that lupeol may exacerbate MT1-MMP-mediated NF-κB signaling under pro-inflammatory stimulation and within highly MT1-MMP expressing tumor cells, while anti-inflammatory actions within normal-to-low MT1-MMP expressing tumor cells may classically elicit lupeol's pharmacological effects in lowering inflammation biomarkers (Fig. 5).

Accordingly, we also highlight specific, yet not fully elucidated, transcriptional regulation properties of lupeol against MT1-MMP since lupeol did not affect neither of PMA-induced COX-2 nor MMP-9 gene expression. Interestingly, while attenuation of MT1-MMP expression was reported to decrease TGF-β1-mediated MMP-9 expression, we further report that similar transcriptional regulation may possibly also occur in PMA-treated MT1-MMP-invalidated cells. Altogether, this evidence confirms the requirement of some MT1-MMP signaling axis in MMP-9 expression regulation. Evidence that MT1-MMP activates a number of intracellular signal pathways including the extracellular signal-related kinase (ERK) pathway, the focal adhesion

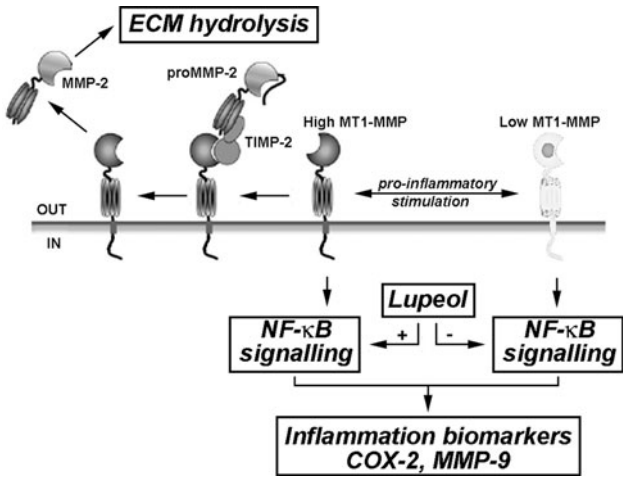

Fig. 5 MT1-MMP expression levels regulate lupeol's pharmacological actions. Pro-inflammatory stimulation (such as with PMA) triggers NF-κB signaling within high or normal-to-low MT1-MMP expressing cells. While pro-inflammatory stimulation also elicits MT1-MMP-mediated proMMP-2 activation that leads to extracellular matrix (ECM) hydrolysis and cell invasion, lupeol may exert a dual role as it is found to exacerbate (*plus symbol*) MT1-MMP signaling that leads to increased expression of inflammation biomarkers. In contrast, lupeol may inhibit (*minus symbol*) NF-κB signaling in low MT1-MMP expressing cells

kinase (FAK), Src, RhoA/ROK, Rac and NF-κB pathways has been reported (Takino et al. 2004; Annabi et al. 2005; Sato et al. 2005; Sounni and Noel 2005; Annabi et al. 2009). Although not statistically significant, MMP-9 gene expression was also slightly abrogated by lupeol in those PMA-treated MT1-MMP-invalidated cells. Thereof, such unusual signaling contribution of MT1-MMP included a role in PGE_2-induced angiogenesis (Alfranca et al. 2008), platelet-mediated calcium mobilization (Fortier et al. 2008), regulation of cell death/survival bioswitch (Belkaid et al. 2007; Fortier et al. 2008), and radioresistance in both glioma cells (Wild-Bode et al. 2001; Wick et al. 2002) and endothelial cells (Annabi et al. 2003). In addition to signal transduction, some cellular contribution was also ascribed to MT1-MMP through the recent demonstration that it also played a role in medulloblastoma CD133(+) neurosphere-like formation and increased invasiveness (Annabi et al. 2009). Paradoxical molecular and cellular impact of MT1-MMP reinforces the concept of assessing the pharmacological properties of a given agent on alternative pathways. Recent COX inhibitors, for instance, have been shown to rather directly alter gene and protein expression of specific targets involved in tumorigenesis and inflammation (Wang et al. 2011).

Pharmacological targeting of NF-κB-mediated MMP-9 expression was recently reported by us in DAOY medulloblastoma-derived cells and in human brain microvascular endothelial cells (Annabi et al. 2008b, 2009c, 2010). In the current study, we now demonstrate that MT1-MMP-mediated signaling, in part, contributes to the transcriptional regulation of MMP-9 expression upon lupeol/PMA stimulation, and that this possibly involved stabilization of the 3′UTR site of the MMP-9 transcript by the ELAV-like protein HuR. Insufficient HuR-mediated transcript stabilization may ultimately result in the rapid degradation of the MMP-9 transcript (Fan and Steitz 1998). Moreover, it should be acquainted that HuR synthesis is, in part, regulated by NF-κB signaling (Kang et al. 2008), further supporting that HuR downregulated expression upon silencing of the MT1-MMP-mediated signaling axis may significantly impact on its contribution to MMP-9 expression. As basal HuR protein levels remained unaffected by lupeol treatment in our MT1-MMP knockout conditions, this experimental evidence confirms that lupeol may bear dual pharmacological actions which can only be revealed under specific in vitro culture conditions that mimic inflammation.

In conclusion, this study is among the first in vitro investigation to report lupeol's mechanism of action under pro-inflammatory stimulation. Aside from its apparent protection against PMA-induced superoxide generation in human neutrophils (Yamashita et al. 2002), we provide new evidence that multiple signaling pathways may be involved in the lupeol regulation of inflammatory biomarkers gene expression such as MMP-2, MMP-9 and COX-2. Intracellular signaling cascades mediated by MT1-MMP, possibly through its intracellular cytoplasmic domain, will remain to be further investigated but seem to play a key role in lupeol's effects under such conditions. Whether similar mechanisms are also involved in the actions of other dietary phytochemicals is currently unknown. Based upon our results, unsuspected and dual pharmacological functions of anti-inflammatory agents may, under specific experimental conditions, possibly exacerbate neoplasic adaptative mechanisms and limit therapeutic efficacy. Given that adult and pediatric intracranial tumors are characterized by increased extravasation and infiltration of lymphocytes into regions of chronic local inflammation, design of alternate and more optimal therapeutic strategies based on targeting microenvironment-mediated chronic inflammation and angiogenesis will be required.

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References

Alfranca A, López-Oliva JM, Genís L et al (2008) PGE2 induces angiogenesis via MT1-MMP-mediated activation of the TGFb-eta/Alk5 signaling pathway. Blood 112:1120–1128

Amalinei C, Caruntu ID, Giusca SE et al (2010) Matrix metalloproteinases involvement in pathologic conditions. Rom J Morphol Embryol 51:215–228

Annabi B, Lee YT, Martel C et al (2003) Radiation induced-tubulogenesis in endothelial cells is antagonized by the antiangiogenic properties of green tea polyphenol (-) epigallocatechin-3-gallate. Cancer Biol Ther 2:642–649

Annabi B, Bouzeghrane M, Moumdjian R et al (2005) Probing the infiltrating character of brain tumors: inhibition of RhoA/ROK-mediated CD44 cell surface shedding from glioma cells by the green tea catechin EGCg. J Neurochem 94:906–916

Annabi B, Rojas-Sutterlin S, Laflamme C et al (2008a) Tumor environment dictates medulloblastoma cancer stem cell expression and invasive phenotype. Mol Cancer Res 6:907–916

Annabi B, Rojas-Sutterlin S, Laroche (2008b) The diet-derived sulforaphane inhibits matrix metalloproteinase-9-activated human brain microvascular endothelial cell migration and tubulogenesis. Mol Nutr Food Res 52:692–700

Annabi B, Lachambre MP, Plouffe K et al (2009a) Modulation of invasive properties of CD133+ glioblastoma stem cells: a role for MT1-MMP in bioactive lysophospholipid signaling. Mol Carcinog 48:910–919

Annabi B, Laflamme C, Sina A et al (2009b) A MT1-MMP/NF-kappaB signaling axis as a checkpoint controller of COX-2 expression in CD133+U87 glioblastoma cells. J Neuroinflammation 6:8

Annabi B, Lachambre MP, Plouffe K et al (2009c) Propranolol adrenergic blockade inhibits human brain endothelial cells tubulogenesis and matrix metalloproteinase-9 secretion. Pharmacol Res 60:438–445

Annabi B, Vaillancourt-Jean E, Weil AG et al (2010) Pharmacological targeting of beta-adrenergic receptor functions abrogates NF-kappaB signaling and MMP-9 secretion in medulloblastoma cells. Onco Targets Ther 3:219–226

Bao S, Wu Q, McLendon RE et al (2006) Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 444:756–760

Béliveau R, Gingras D (2007) Role of nutrition in preventing cancer. Can Fam Phys 53:1905–1911

Belkaid A, Fortier S, Cao J et al (2007) Necrosis induction in glioblastoma cells reveals a new "bioswitch" function for the MT1-MMP/G6PT signaling axis in proMMP-2 activation versus cell death decision. Neoplasia 9:332–340

Bu X, Zhao C, Dai X (2011) Involvement of COX-2/PGE(2) pathway in the upregulation of MMP-9 expression in pancreatic cancer. Gastroenterol Res Pract 2011:214269

Chambers AF, Matrisian LM (1997) Changing views of the role of matrix metalloproteinases in metastasis. J Natl Cancer Inst 89: 1260–1270

Currie JC, Fortier S, Sina A et al (2007) MT1-MMP down-regulates the glucose 6-phosphate transporter expression in marrow stromal cells: a molecular link between pro-MMP-2 activation, chemotaxis, and cell survival. J Biol Chem 282:8142–8149

Fan XC, Steitz JA (1998) Overexpression of HuR, a nuclear-cytoplasmic shuttling protein, increases the in vivo stability of ARE-containing mRNAs. EMBO J 17:3448–3460

Fernandez MA, de lasHeras B, Garcia MD et al (2001) A new insights into the mechanism of action of the anti-inflammatory triterpene lupeol. J Pharm Pharmacol 53:1533–1539

Fortier S, Labelle D, Sina A et al (2008a) Silencing of the MT1-MMP/G6PT axis suppresses calcium mobilization by sphingosine-1-phosphate in glioblastoma cells. FEBS Lett 582:799–804

Fortier S, Touaibia M, Lord-Dufour S et al (2008b) Tetra- and hexavalent mannosides inhibit the pro-apoptotic, antiproliferative and cell surface clustering effects of concanavalin-A: impact on MT1-MMP functions in marrow-derived mesenchymal stromal cells. Glycobiology 18:195–204

Geetha T, Varalakshmi P (1999) Anticomplement activity of triterpenes from *Crataeva nurvala* stem bark in adjuvant arthritis in rats. Gen Pharmacol 32:495–497

Han YP, Tuan TL, Wu H et al (2001) TNF-alpha stimulates activation of pro-MMP2 in human skin through NF-(kappa)B mediated induction of MT1-MMP. J Cell Sci 114:131–139

Kang MJ, Ryu BK, Lee MG et al (2008) NF-kappaB activates transcription of the RNA-binding factor HuR, via PI3K-AKT signaling, to promote gastric tumorigenesis. Gastroenterology 135:2030–2042

Kanu OO, Mehta A, Di C et al (2009) Glioblastoma multiforme: a review of therapeutic targets. Expert Opin Ther Targets 13: 701–718

Mazloom A, Zangeneh AH, Paulino AC (2010) Prognostic factors after extraneural metastasis of medulloblastoma. Int J Radiat Oncol Biol Phys 78:72–78

Moreau RA, Whitaker BD, Hicks KB (2002) Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. Prog Lipid Res 41:457–500

Ou Y, Li W, Li X et al (2011) Sinomenine reduces invasion and migration ability in fibroblast-like synoviocytes cells co-cultured with activated human monocytic THP-1 cells by inhibiting the expression of MMP-2, MMP-9, CD147. Rheumatol Int 31: 1479–1485

Packer RJ (2007) Craniospinal radiation therapy followed by adjuvant chemotherapy for newly diagnosed average-risk medulloblastoma. Curr Neurol Neurosci Rep 7:130–132

Papi Reddy K, Singh AB, Puri A, Srivastava AK, Narender T (2009) Synthesis of novel triterpenoid (lupeol) derivatives and their in vivo antihyperglycemic and antidyslipidemic activity. Bioorg Med Chem Lett 19:4463–4466

Proulx-Bonneau S, Pratt J, Annabi B (2011) A role for MT1-MMP as a cell death sensor/effector through the regulation of endoplasmic reticulum stress in U87 glioblastoma cells. J Neurooncol 104:33–43

Saleem M (2009) Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene. Cancer Lett 285:109–115

Saleem M, Afaq F, Adhami VM et al (2004) Lupeol modulates NF-kappaB and PI3K/Akt pathways and inhibits skin cancer in CD-1 mice. Oncogene 23:5203–5214

Saleem M, Kaur S, Kweon MH et al (2005) Lupeol, a fruit and vegetable based triterpene, induces apoptotic death of human pancreatic adenocarcinoma cells via inhibition of Ras signaling pathway. Carcinogenesis 26:1956–1964

Sato H, Takino T, Miyamori H (2005) Roles of membrane-type matrix metalloproteinase-1 in tumor invasion and metastasis. Cancer Sci 96:212–217

Siddique HR, Saleem M (2011) Beneficial health effects of lupeol triterpene: a review of preclinical studies. Life Sci 88:285–293

Sina A, Proulx-Bonneau S, Roy A et al (2010) The lectin concanavalin-A signals MT1-MMP catalytic independent induction of COX-2 through an IKKγ/NF-κB-dependent pathway. J Cell Commun Signal 4:31–38

Sounni NE, Noel A (2005) Membrane type-matrix metalloproteinases and tumor progression. Biochimie 87:329–342

Sudhahar V, Kumar SA, Varalakshmi P (2006) Role of lupeol and lupeol linoleate on lipemic-oxidative stress in experimental hypercholesterolemia. Life Sci 78:1329–1335

Sudhahar V, Kumar SA, Sudharsan PT et al (2007) Protective effect of lupeol and its ester on cardiac abnormalities in experimental hypercholesterolemia. Vasc Pharmacol 46:412–418

Tahanian E, Peiro S, Annabi B (2011a) Low intracellular ATP levels exacerbate carcinogen-induced inflammatory stress response and inhibit in vitro tubulogenesis in human brain endothelial cells. J Inflamm Res 4:1–10

Tahanian E, Sanchez LA, Shiao TC et al (2011b) Flavonoids targeting of IκB phosphorylation abrogates carcinogen-induced MMP-9 and COX-2 expression in human brain endothelial cells. Drug Des Dev Ther 5:299–309

Takino T, Miyamori H, Watanabe Y et al (2004) Membrane type 1 matrix metalloproteinase regulates collagen-dependent mitogen-activated protein/extracellular signal-related kinase activation and cell migration. Cancer Res 64:1044–1049

Tarapore RS, Siddiqui IA, Saleem M et al (2010) Specific targeting of Wnt/beta-catenin signaling in human melanoma cells by a dietary triterpene lupeol. Carcinogenesis 31:1844–1853

Wang X, Baek SJ, Eling T (2011) COX inhibitors directly alter gene expression: role in cancer prevention? Cancer Metastasis Rev 30:641–657

Wick W, Wick A, Schulz JB et al (2002) Prevention of irradiation-induced glioma cell invasion by temozolomide involves caspase 3 activity and cleavage of focal adhesion kinase. Cancer Res 62:1915–1919

Wild-Bode C, Weller M, Rimner A et al (2001) Sublethal irradiation promotes migration and invasiveness of glioma cells: implications for radiotherapy of human glioblastoma. Cancer Res 61: 2744–2750

Yamashita K, Lu H, Lu J et al (2002) Effect of three triterpenoids, lupeol, betulin, and betulinic acid on the stimulus-induced superoxide generation and tyrosyl phosphorylation of proteins in human neutrophils. Clin Chim Acta 325:91–96