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Biophysical evidence for differential gallated green tea catechins binding to membrane type-1 matrix metalloproteinase and its interactors

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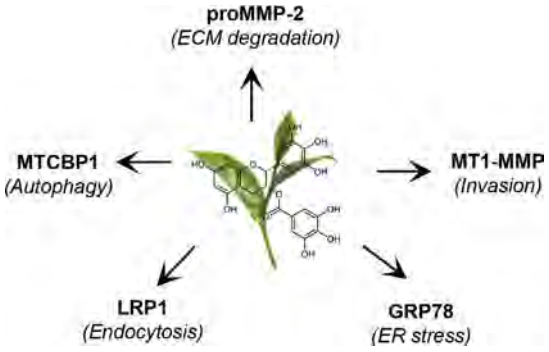
HIGHLIGHTS

- Multiple tumorigenic processes involve membrane type-1 matrix metalloproteinase.
- Disease prevention properties of tea have been attributed to catechins.
- Surface plasmon resonance was used to assess catechins binding to MT1-MMP.
- Gallated catechins have higher affinity towards MT1-MMP and its interactors.

GRAPHICAL ABSTRACT



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ABSTRACT

Membrane type-1 matrix metalloproteinase (MT1-MMP) is a transmembrane MMP which triggers intracellular signaling and regulates extracellular matrix proteolysis, two functions that are critical for tumor-associated angiogenesis and inflammation. While green tea catechins, particularly epigallocatechin gallate (EGCG), are considered very effective in preventing MT1-MMP-mediated functions, lack of structure-function studies and evidence regarding their direct interaction with MT1-MMP-mediated biological activities remain. Here, we assessed the impact in both cellular and biophysical assays of four ungallated catechins along with their gallated counterparts on MT1-MMP-mediated functions and molecular binding partners. Concanavalin-A (ConA) was used to trigger MT1-MMP-mediated proMMP-2 activation, expression of MT1-MMP and of endoplasmic reticulum stress biomarker GRP78 in U87 glioblastoma cells. We found that ConA-mediated MT1-MMP induction was inhibited by EGCG and catechin gallate (CG), that GRP78 induction was inhibited by EGCG, CG, and gallocatechin gallate (GCG), whereas proMMP-2 activation was inhibited by EGCG and GCG. Surface plasmon resonance was used to assess direct interaction between catechins and MT1-MMP interactors. We found that gallated catechins interacted better than their ungallated analogs with MT1-MMP as well as with MT1-MMP binding partners MMP-2, TIMP-2, MTCBP-1 and LRP1-clusterIV. Overall, current structure-function evidence supports a role for the galloyl moiety in both direct and indirect interactions of green tea catechins with MT1-MMP-mediated oncogenic processes.

Abbreviations: C, catechin; CNS, central nervous system; ConA, concanavalin-A; EC, epicatechin; ECM, extracellular matrix; EGC, epigallocatechin; EGCg, epigallocatechin gallate; GBM, glioblastoma; 67LR, 67 k kDa laminin receptor; LRP-1, low-density-lipoprotein receptor-related protein-1; MMP-2, metalloproteinases-2; MT1-MMP, membrane type-1 matrix metalloproteinase; MTCBP-1, MT1-MMP cytoplasmic tail-binding protein-1; SPR, surface plasmon resonance; TIMP-2, tissue inhibitor of metalloproteinase-2; TLR, Toll-like receptor

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1. Introduction

The use of various natural phytochemicals and dietary compounds in cancer chemoprevention is believed to prevent the onset of pathophysiological processes which regulate tumor growth [1]. Furthermore, epidemiological and pre-clinical data, obtained through *in vitro* and *in vivo* animal studies, support the concept that these compounds can downregulate oncogenic signaling pathways or sensitize malignant cells to cytotoxic agents [2]. Unfortunately, only a limited number of these compounds have been tested in clinical trials often because of the lack of structure-function molecular evidence supporting their capacity to interact with their cellular targets and to interfere with the associated biological processes [3].

Among natural polyphenolic compounds, prophylactic and therapeutic properties have been attributed to green tea catechins and black tea theaflavins [4]. In fact, among tea polyphenols which possess potent antioxidant and anti-inflammatory properties that modulate signaling pathways [5], epigallocatechin-3-gallate (EGCG) is one of the most studied active substances and considered to act through diversified molecular mechanisms [6]. Its *in vitro* cellular effects were documented in numerous central nervous system (CNS) cancer cell models including glioblastoma [7–9], pediatric brain tumor-derived medulloblastoma [10], and in childhood primitive neuroectodermal brain tumors [11]. Interestingly, combining EGCG with Temozolomide [12] or to ionizing radiation [13, 14] was found to enhance therapeutic efficacy. Among the molecular processes targeted by EGCG, inhibition of cell proliferation, survival, *in vitro* endothelial cell tubulogenesis, pro-inflammatory intracellular transducing events, as well as cell migration/invasion processes have been reported [15, 16]. In addition, EGCG was recently documented to alter the membrane bound matrix metalloproteinase MT1-MMP functions in cancer cell invasion and survival processes through the inhibition of its capacity to hydrolyze extracellular matrix

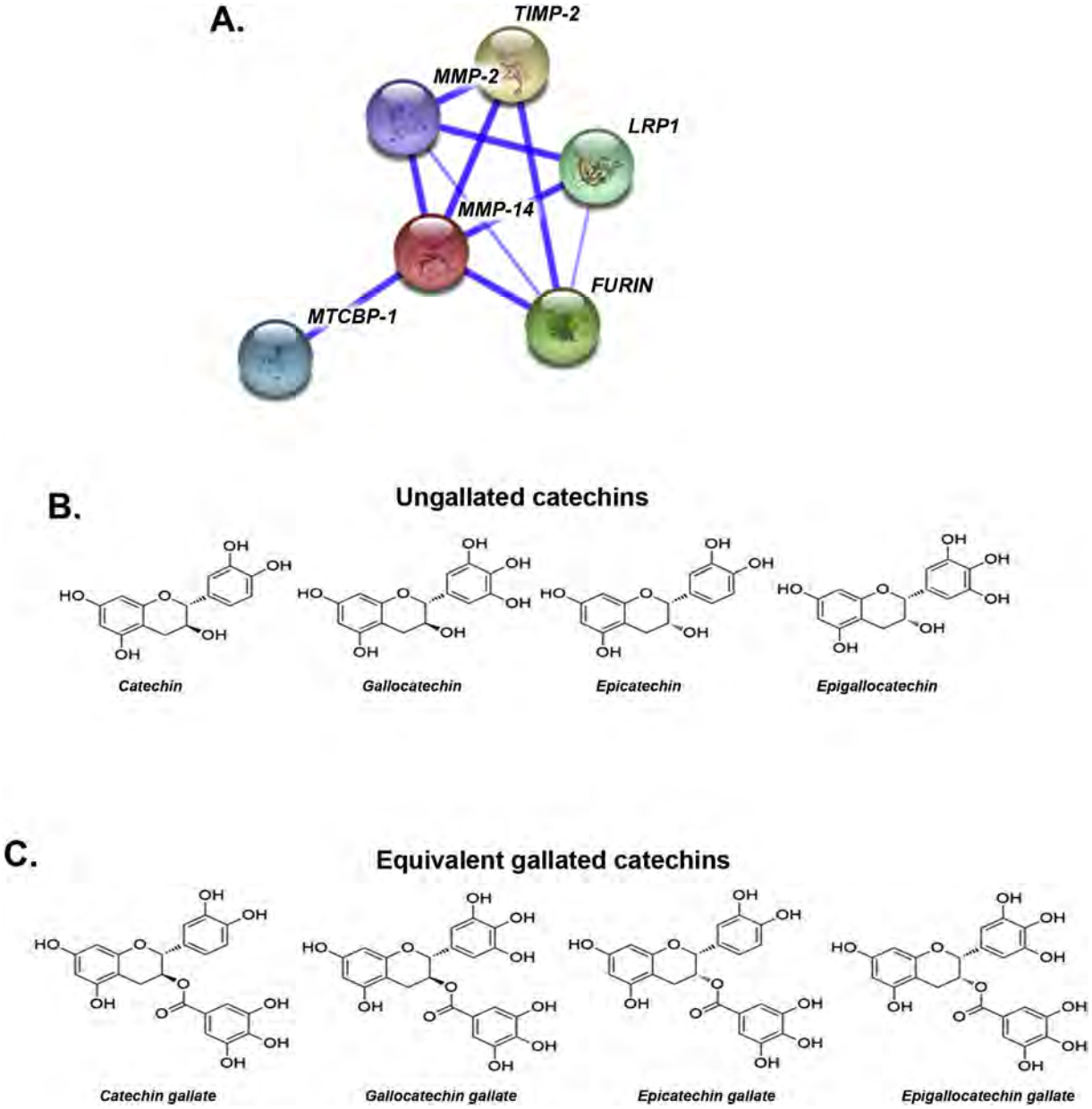

Fig. 1. Scheme of MT1-MMP predicted interactors and molecular structure of green tea-derived catechins. A) STRING V10.0 algorithm was used to identify MT1-MMP protein-protein interactors (http://string-db.org/). LRP-1, Low density lipoprotein receptor-related protein 1, MT1-MMP, membrane type-1 matrix metalloproteinase; MMP-2, matrix metalloproteinase-2, MTCBP-1, MT1-MMP cytoplasmic tail-binding protein-1; TIMP, tissue inhibitor of matrix metalloproteinase. Chemical structures of B) ungallated catechins, and C) equivalent gallated catechins which were used in cellular and acellular assays. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

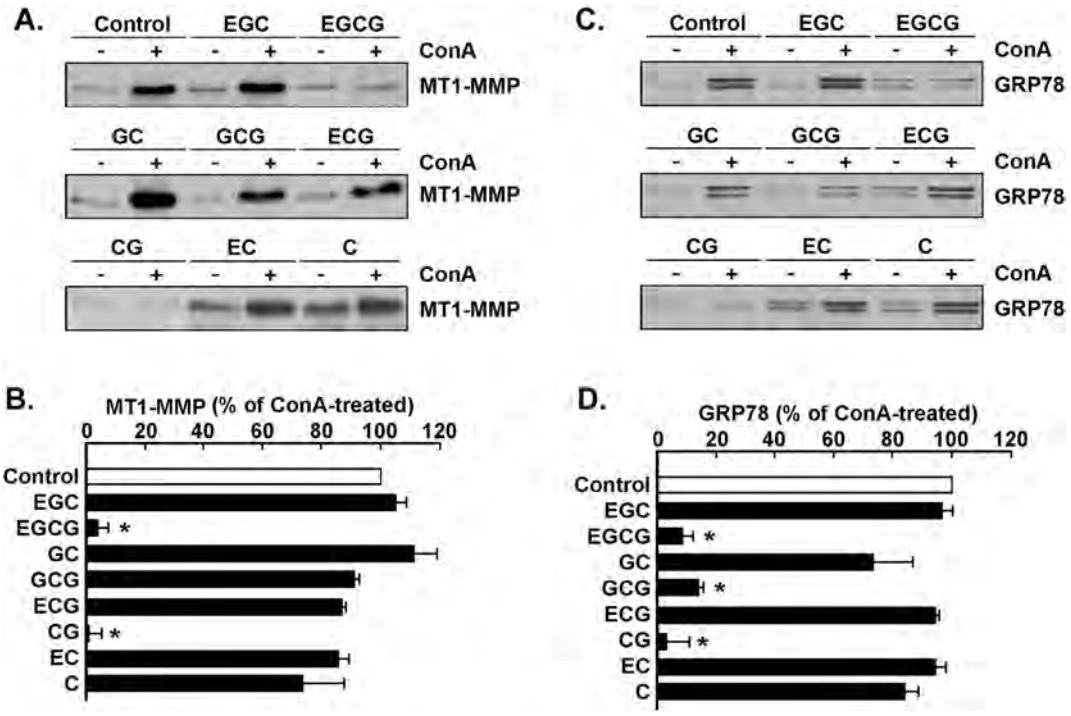

Fig. 2. Impact of catechins on Concanavalin-A-induced MT1-MMP expression in U87 glioblastoma cells. Serum-starved U87 glioblastoma cells were treated with 30 μg/ml ConA for 24 h in the presence of 30 μM of each of either the ungallated catechins (C, GC, EC, EGC) or with equivalent gallated catechins (CG, GCG, ECG, EGCG). Lysates were harvested upon treatment and immunoblots performed for A) MT1-MMP and C) GRP78 as described in the Materials and methods section. Scanning densitometry was used to quantify the extent of ConA-mediated B) MT1-MMP and D) GRP78 inductions.

(ECM) components [17], to activate latent proMMP [18], and to signal pro-angiogenic and pro-inflammatory intracellular events involving Erk, RhoA/ROK, Src or JAK/STAT3 pathways [8, 19, 20].

While evidence suggests that the effects of EGCG are, in part, mediated through its specific interaction with the 67 kDa laminin receptor (67LR) [21], the molecular mechanism of action of the other catechins remains mostly unknown. Interestingly, interaction studies of catechins with the 67LR has led to the conclusion that catechin (C), epicatechin (EC), and epigallocatechin (EGC) were unable to bind to the 67LR [21]. Unfortunately, much less information was provided about the gallated catechins. It is yet unknown whether EGCG's structural analogs, that also contain the galloyl moiety, bind to the same EGCG receptor. Initial structure-function studies have suggested galloyl moiety of catechins to inhibit phorbol ester-induced MMP-9 and HuR expressions [22], as well as to dictate fatty-acid synthase inhibition [23]. More recently, *in silico* molecular docking analysis predicted EGCG and epicatechin-3-gallate (ECG) mode of binding to MT1-MMP and proMMP-2/MMP-2 [24], as well as to proMMP-9/MMP-9 [25]. However, these studies were conducted at supraphysiological millimolar concentrations of catechins and did not assess the ability of catechins to alter MT1-MMP's intracellular signaling capacities. Whether physiological plasmatic concentrations of any of these catechins allow direct binding and interaction with MT1-MMP itself or interactors of MT1-MMP has not been documented yet.

In the current study, we used surface plasmon resonance (SPR) and compared the potential direct interaction of four ungallated green tea-derived catechins to their gallated equivalents towards either MT1-MMP itself, to several predicted interactors such as matrix metalloproteinase-2 (MMP-2), tissue inhibitor of metalloproteinases (TIMP)-2, MT1-MMP cytoplasmic tail-binding protein-1 (MTCBP-1), or to MT1-MMP cell surface substrates such as the low-density-lipoprotein receptor-related protein-1 (LRP-1). Structure-function cellular assays were also performed at physiological plasmatic catechin concentrations to address MT1-MMP-dependent processes involved in proMMP-2 activation and in GRP78 endoplasmic reticulum stress induction within ConA-activated U87 glioblastoma cells.

2. Results

2.1. MT1-MMP interactors and structure of several green tea-derived catechins

MT1-MMP possesses extracellular hydrolytic activities as well as intracellular cell transducing functions in brain tumorigenesis [26]. Among the principal molecular interactors predicted using the STRING protein-protein interaction database [27], MT1-MMP, proMMP-2 (MMP-2) and TIMP-2 were confirmed and are well known to contribute to the formation of the ternary complex required for optimal processing of latent proMMP-2 into active MMP-2 (Fig. 1A) [28]. TIMP-1 was also identified (not shown) but is known to weakly interact with MT1-MMP [29, 30]. The cell surface low density lipoprotein-related protein 1 (LRP1), as well as the intracellular partners Furin and MTCBP-1 are believed to interact with the cytoplasmic domain of MT1-MMP, which interaction could possibly modulate MT1-MMP's signal transducing functions [31]. In order to document the possible structure-function interactions of catechins with these predicted interactors, the structure of four ungallated catechins (Fig. 1B) as well as of four gallated equivalent catechins (Fig. 1C) were first tested on known cellular conditions which require MT1-MMP functions.

2.2. Cellular impact of catechins on Concanavalin-A-induced expressions of MT1-MMP and GRP78, and on the activation of proMMP-2

Despite the evidence supporting green tea catechins possess chemopreventive and anti-inflammatory properties [32], structure-function studies remain scarce. We decided to treat serum-starved U87 glioblastoma cells with Concanavalin-A (ConA), a well-known *in vitro* inducer of MT1-MMP [33, 34] (Fig. 2A) as well as of inflammation [35, 36] and autophagy [37, 38], in the presence or absence of eight different catechins. We found that EGCG and CG were the most potent

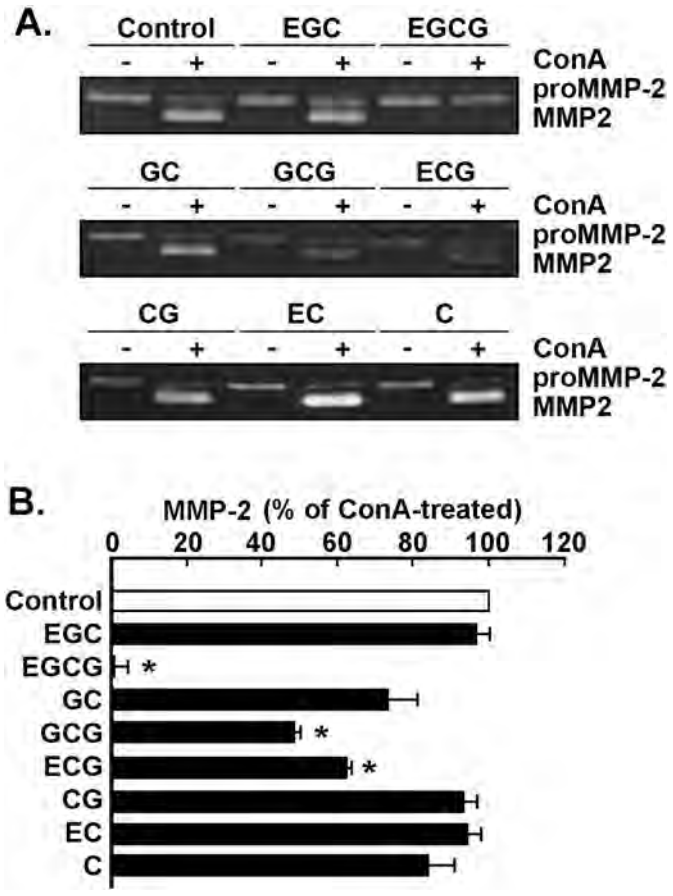

Fig. 3. Impact of catechins on Concanavalin-A-induced proMMP-2 activation in U87 glioblastoma cells. Serum-starved U87 glioblastoma cells were treated with 30 μg/ml ConA for 24 h in the presence of 30 μM of each of either the ungallated catechins (C, GC, EC, EGC) or with gallated catechins (CG, GCG, ECG, EGCG). A) Conditioned media were harvested upon treatment and gelatin zymography performed as described in the Materials and methods section. B) Scanning densitometry of the zymograms was used to quantify the extent of ConA-mediated proMMP-2 activation.

catechins to inhibit ConA-mediated induction of MT1-MMP (Fig. 2B). A slight non-statistically significant inhibitory effect (12–15%) was also observed with GCG, ECG, EC, and C (Fig. 2B). When ConA-mediated induction of the inflammation and endoplasmic reticulum stress biomarker GRP78 expression was monitored (Fig. 2C), we found that EGCG, GCG, and CG were the most potent inhibitory catechins (Fig. 2D). Finally, we further monitored the impact of catechins upon ConA-mediated proMMP-2 activation (Fig. 3A), a process believed to require the formation of a ternary MT1-MMP/TIMP-2/proMMP-2 complex [39]. We found that EGCG, ECG and GCG were the most efficient catechins to prevent proMMP-2 activation as assessed by zymography of the respective condition media harvested (Fig. 3B). Collectively, we conclude that only those catechins specifically bearing the galloyl moiety efficiently altered either ConA-mediated intracellular signaling that regulates MT1-MMP and GRP78 expression, or MT1-MMP-mediated activation of proMMP-2 within the extracellular compartment of U87 glioblastoma cells. Whether any of these gallated catechins could directly bind to MT1-MMP or to its interactors was next assessed.

2.3. Binding assessment of gallated catechins to MT1-MMP and its interactors using SPR

In order to assess the biophysical properties (capacity and affinity to bind to immobilized proteins) of gallated catechins, we hypothesized that they could directly target MT1-MMP or some of its interactors. Thus, through SPR biosensor technology, we first evaluated the binding of four gallated catechins to MT1-MMP immobilized on CM5 chips. Representative sensograms show that the four tested gallated catechins significantly interacted with MT1-MMP, with EGCG demonstrating the highest level of binding among them (Fig. 4A). In sharp contrast, none of the tested ungallated catechins demonstrated significant interaction with MT1-MMP (sensograms not shown, refer to Kd constants in Fig. 5). When catechin binding was evaluated onto immobilized MMP-2 (Fig. 4B) or onto immobilized TIMP-2 (Fig. 4C) proteins, we observed that the gallated catechins again targeted significantly those MT1-MMP interactors that collectively contribute to the *in vivo* formation of the MT1-MMP/MMP-2/TIMP-2 ternary complex. Interestingly, MTCBP-1 was also found to significantly be targeted by gallated catechins (Fig. 4D), as well as the MT1-MMP cell surface substrate LRP-1 (Fig. 4E). Collectively, these observations suggest that potential hierarchical catechin binding processes could potentiate the combined actions of those gallated catechins. Whether, their binding interferes sequentially with either of these interactors during the formation of the ternary complex remains to be determined.

3. Discussion

In this study, we assessed for the first time the direct biophysical binding capacities of green tea-derived catechins at physiological concentrations to MT1-MMP and to MT1-MMP interactors. Our study further emphasizes on the importance of the galloyl moiety, which affects the *in vitro* interacting ability of catechins towards MT1-MMP-mediated proMMP-2 activation processes. In addition, we ranked the catechins according to their structures and binding affinities towards MT1-MMP and MT1-MMP interactors (Fig. 5). Such affinity ranking suggests a hierarchical targeting from the catechins tested and further confirms their potential synergistic molecular action as they are found in food, and explain their pleiotropic anticancer properties against cell signaling functions.

Aside from its canonical role in ECM proteolysis, targeting strategies recently rather aim at altering MT1-MMP's involvement in crucial intracellular signal transduction that control several processes related to cell mobilization and survival [40–42]. As such, it was demonstrated that, as a consequence of ConA-mediated MT1-MMP activation, sequential Src kinase and JAK/STAT3 signaling were required to upregulate CSF-2 and CSF-3 transcription [20]. In that study, the anti-angiogenic EGCG was able to abrogate both ConA- and MT1-MMP-induced JAK/STAT signaling, which effect adds-up to EGCG's pleiotropic biophysical properties. Catechins do not behave exactly as synthetic drugs since they are polytarget molecules given their final effect is due to multiple elements adding up. Direct physical interaction between catechins and membrane-imbedded molecules may, therefore, only represent part of their chemopreventive effects as, for instance, destabilization of lipid rafts or changes in membrane mechanics have recently been inferred [43].

Since its discovery, MT1-MMP has been extensively investigated and emerged among the best characterized MMPs. MT1-MMP is involved in various physiological and pathological processes such as wound healing, bone development, angiogenesis, inflammation, cancer invasion and metastasis [44]. Formation of a ternary MMP-2/TIMP-2/MT1-MMP complex at the cell surface is now a well-recognized mechanism to trigger ECM hydrolysis [45, 46], and MT1-MMP primary functions immediately outside the cell surface [47] believed to drive signaling either from ECM adhesion molecules or in response to chemokines [47, 48]. Given that all three molecules, MMP-2, TIMP-2 and MT1-MMP, have demonstrated the capacity to interact with gallated catechins, a plausible mechanism of action of these catechins may be, prior to extracellular complexation of MMP-2/TIMP-2/MT1-MMP, to hierarchically bind to them and respectively trigger conformational changes in their structure that would alter ability to lead to ECM degradation or to relay intracellular signals. To validate the latter, one

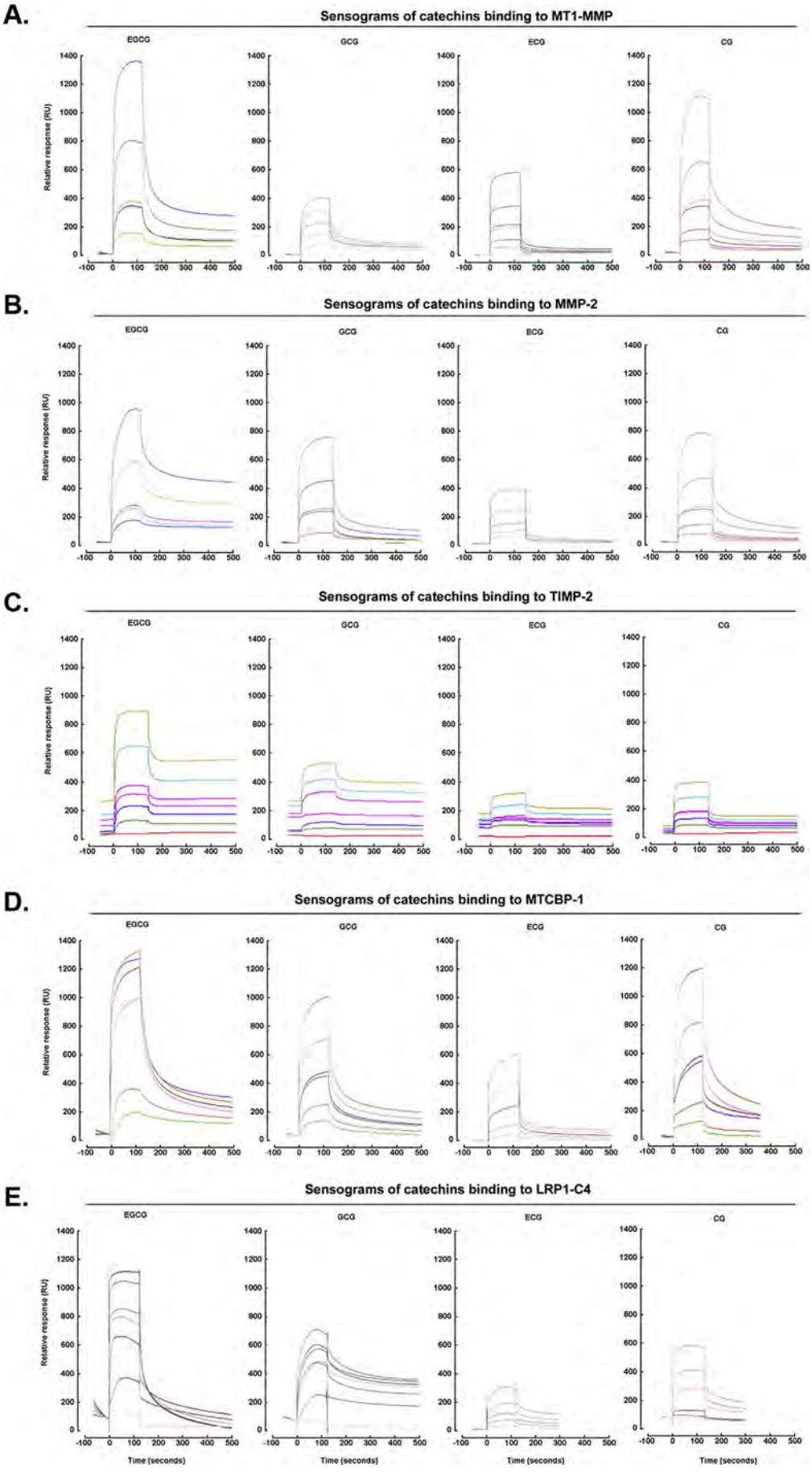

Fig. 4. Binding assessment of catechins to MT1-MMP and its interactors by SPR. Recombinant A) MT1-MMP, B) MMP-2, C) TIMP-2, D) MTCBP-1, or E) LRP1-cluster IV (LRP1-C4) were immobilized on CM5 sensor chips and increasing amounts (0–100 μM) of different gallated catechins assessed for interaction by SPR as described in the Materials and methods section. Representative sensograms are shown.

A.

	Kd (nM)				
	MT1-MMP	MMP-2	TIMP-2	MTCBP-1	LRP1
GCG	220	380	52	74	9.5
EGCG	95	251	47	4.1	1.5
EGC	297	135	3	149	176
GC	n.d.	98	86	44	n.d.
C	293	n.d.	21	n.d.	285
CG	250	306	47	62	102
ECG	256	261	76	86	165
EC	n.d.	470	10	n.d.	n.d.

B.

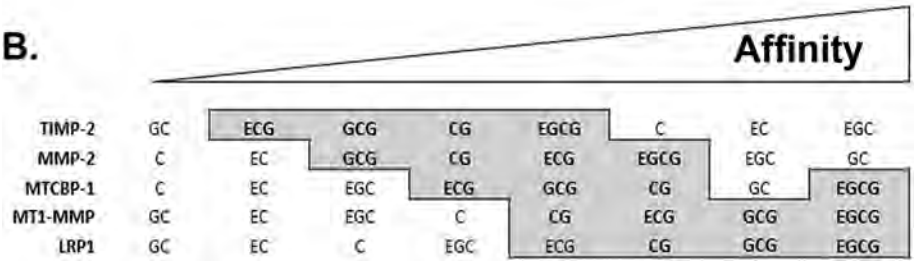

Fig. 5. Kd constants and catechins affinity ranking towards MT1-MMP interactors. Some of recombinant MT1-MMP interactors were immobilized on CM5 sensor chips and increasing amounts (0–100 μM) of different catechins assessed for interaction by SPR as described in the Materials and methods section. A) Kd constants were derived from representative sensograms, and B) affinity ranking towards the immobilized proteins depicted from catechins with low affinity to high affinity catechins. The gallated catechins were grouped into shadowed grey.

would need a complex structure-to-function study with MT1-MMP deletion mutants within its catalytic, hemopexin, and hinge domains all believed to recognize, anchor, and catalyze proMMP to MMP activation. Tentative modeling studies of gallated catechins interaction with MT1-MMP, proMMP-2 and MMP-2 were recently reported and some predicted residues highlighted [23b]. The relevance of these residues in gallated catechins binding still awaits confirmation through site-directed mutagenesis studies.

Our current study also sheds light on the emerging importance of cell surface modulators such as the naturally occurring plant lectin Concanavalin-A (ConA). Such lectin is used by the pharmaceutical industry as part of a reliable *in vitro* cell functional assay for studying biological systems ranging from mitogenicity to pro-inflammatory cytokine production [49, 50]. Given the recent use of the lectin ConA, a potent Toll-Like receptors TLR-2 and TLR-6 agonist [20], our study thus supports the potential green tea catechins-mediated targeting of existing crosstalk between TLRs and cancer [51], angiogenesis [52] and inflammation [53]. Interestingly, ConA is well known to also induce MT1-MMP, the expression of which has also been documented in all the above-regulated processes, similarly to TLR involvement [31, 54]. Our current cellular and acellular approaches imply that EGCG inhibits both ConA- and MT1-MMP-mediated signaling. In support of this, EGCG was already described as inhibiting TLR-2 and TLR-4 signaling [55, 56], as well as the signaling that leads to diminished MT1-MMP gene expression, and that alter signaling cascades that lead to Src and STAT3 phosphorylation [20, 57]. Besides its impact on cancer progression and angiogenesis, catechins-mediated inhibition of MT1-MMP-regulated gene transcription seems to also be among the functions of catechins that may impact on the expression of biomarkers in autophagy [37] as well as in neuroinflammation [58].

Furthermore, the *in vitro* targeting efficacy of EGCG was recently investigated on the NF-κB-mediated transcriptional regulation of a panel of 89 biomarkers associated with promyelocytic HL-60 differentiation into macrophages. NF-κB-mediated signaling during HL-60 macrophage differentiation was reversed by EGCG, in part through reduced IκB phosphorylation, and led to the inhibition of moderately-to-highly expressed NF-κB gene targets among which the matrix metalloproteinase (MMP)-9 and the cyclooxygenase (COX)-2 [59]. Interestingly, green tea catechins containing a galloyl group in the 3′ position have also been shown to inhibit tissue factor-induced thrombin generation [60], and to differentially regulate platelet aggregation [61]. Noteworthy, whether the 67-kDa laminin receptor (67LR), which is believed to be the EGCG cell surface receptor [62], provides the specificity and transduces the effects of the other catechins containing the galloyl group remains to be confirmed. In light of our results, it is however tempting to hypothesize that the interaction between the galloyl groups in the 3′ position of catechins with the cell surface 67LR would further account for specific catechins transport, clearance and plasmatic half-life, or downstream signaling modulation.

In conclusion, provided that we report the affinity constant values of EGCG towards MT1-MMP and its interactors to closely approximate its reported plasmatic concentrations of ~1 μM [63, 64], with optimal pharmacological effects achieved at 30 μM, our study puts emphasis on strategies that could aim at targeting MT1-MMP-mediated proangiogenic and immunomodulatory functions of brain cancer cells. We provide the first biophysical evidence that one such strategy may also support the combined chemopreventive properties of diet-derived gallated catechins which pharmacological targeting of the MT1-MMP extracellular and intracellular signaling functions could decrease brain tumor invasiveness.

4. Materials and methods

4.1. Materials

Sodium dodecylsulfate (SDS) and bovine serum albumin (BSA) were purchased from Sigma (Oakville, ON). Cell culture media was obtained from Invitrogen (Burlington, ON). Electrophoresis reagents were purchased from Bio-Rad (Mississauga, ON). The enhanced chemiluminescence (ECL) reagents were from Amersham Pharmacia Biotech (Baie d'Urfé, QC). Micro bicinchoninic acid protein assay reagents were from Pierce (Rockford, IL). The rabbit polyclonal antibody against GRP78 was from Santa Cruz Biotechnology (SC-13968, Dallas, TX). The rabbit polyclonal antibody against MT1-MMP was from Chemicon (AB8102, Temecula, CA). Horseradish peroxidase-conjugated donkey anti-rabbit and anti-mouse IgG secondary antibodies were from Jackson ImmunoResearch Laboratories (West Grove, PA). All other reagents, including the green tea-derived catechins tested, were from Sigma-Aldrich Canada. Purity of the catechins tested is as follows: (-)-Catechin (C0567, ≥97% (HPLC)), (-)-Gallocatechin (G6657, ≥98% (HPLC)), (-)-Epicatechin (E4018, ≥98% (HPLC)), (-)-Epigallocatechin (E3768, ≥95% (HPLC)), (-)-Catechin gallate (C0692, ≥98% (HPLC)), (-)-Gallocatechin gallate (G6782, ≥98% (HPLC)), (-)-Epicatechin gallate (E3893, ≥98% (HPLC)), (-)-Epigallocatechin gallate (50,299, ≥97% (HPLC)).

4.2. Cell culture

The human U87 glioblastoma cell line was purchased from American Type Culture Collection (Manassas, VA) and maintained in Eagle's Minimum Essential Medium containing 10% (v/v) calf serum (HyClone Laboratories, Logan, UT), 1 mM sodium pyruvate, 2 mM glutamine, 100 units/ml penicillin and 100 mg/ml streptomycin. Cells were incubated at 37 °C with 95% air and 5% CO_2.

4.3. Immunoblotting procedures

Cells were lysed in a buffer containing 1 mM each of NaF and Na_3VO_4, and 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 0.1% Tween-20 (TBST). Membranes were further washed in TBST and incubated with primary antibodies (1/1000 dilution) in TBST containing 3% BSA and 0.05% NaN_3. The primary antibody was removed by washing with TBST, followed by a 1 h incubation with horseradish peroxidase-conjugated anti-mouse IgG (1/5000 dilution) in TBST containing 5% non-fat dry milk. Immunoreactive material was visualized by enhanced chemiluminescence (Amersham Biosciences, Baie d'Urfé, QC).

4.4. Gelatin zymography

Gelatin zymography was used to assess the extent of proMMP-2 gelatinolytic activity and activation status as previously described [34]. Briefly, an aliquot (20 µl) of the culture medium was suspended in a loading dye (62.5 mM Tris HCl pH 6,8; 10% glycerol; 2% SDS; 0.00625% Bromophenol Blue) and subjected to SDS-PAGE in a gel containing 0.1 mg/ml gelatin, a substrate that is efficiently hydrolyzed by proMMP-2 and MMP-2. 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.

4.5. Surface plasmon resonance (SPR) analysis

SPR analyses were performed using a Biacore T200 instrument (GE Healthcare). LRP-cluster IV, TIMP-2, MMP-2, MT1-MMP, and MTCBP-1 recombinant proteins (Fitzgerald industries international, Acton, MA) were immobilized on a carboxymethylated dextran CM5 sensor chip (GE Healthcare) using an amine-coupling strategy. Briefly, the sensor chip surface was activated with a 1:1 mixture of *N*-hydroxysuccinimide and 3-(*N*,*N*-dimethylamino)-propyl-*N*-ethylcarbondiimide. Recombinant protein solutions (20 µg/ml) were injected at a flow rate of 10 µl/min using HBS-N running buffer (10 mM HEPES, 150 mM NaCl, pH 7.4) to reach a level of immobilization ranging from 5000 to 10,000 RU. Surfaces (protein and reference) were blocked by the injection of an ethanolamine solution. Binding kinetics of catechins over the immobilized recombinant proteins sensor chip (typically 100–500 nM protein per sensor surface) was evaluated in HBS-N buffer at increased concentrations (6.25 to 100 µM) at a flow rate of 20 µl/min. The sensor chip was regenerated by injecting 20 µl of a 10 mM glycine solution, pH 3. Binding sensograms were obtained by subtraction of the reference flow cell (without protein). Experiments were performed in duplicate and data analysis was performed using the BIA evaluation software package (GE Healthcare) and fit to a one-site Langmuir binding model.

4.6. Statistical data analysis

Data are representative of three or more independent experiments. Statistical significance was assessed using GraphPad Prism 5 software with Student's paired *t*-test. $^*P < .05$ was considered significant.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

D.D. designed and performed the experiments, analyzed data, wrote the manuscript. M.I. performed the SPR experiments and analyzed data. S.B. designed experiments, analyzed SPR data. S.L. designed experiments, contributed to writing the manuscript. B.A. designed the experiments, analyzed data, and wrote the manuscript.

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