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Apigenin inhibits TGF-β_1 induced fibroblast-to-myofibroblast transition in human lung fibroblast populations

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Abstract:
Background: Flavonoids are dietary plant compounds suspected to reduce the incidence of chronic diseases in several regions of the world. Due to anti-allergic and anti-inflammatory activities, apigenin (4',5,7,-trihydroxyflavone) is thought to interfere with crucial events in the pathomechanism of asthma. However, the effect of apigenin on TGF-β-induced fibroblast-to-myofibroblast transition (FMT) in human lung fibroblast populations, a key event in asthma progression, has not yet been addressed.
Methods: Primary human bronchial fibroblasts (HBFs) propagated from *ex vivo* bronchial biopsies derived from patients with diagnosed asthma and human embryonic lung IMR-90 fibroblasts were cultured *in vitro* and treated with TGF-β_1 and apigenin. The myofibroblast fraction in fibroblast populations was evaluated by immunocytochemistry. Expression of α-smooth muscle actin (α-SMA) and tenascin C were assessed at the mRNA and protein level by real-time RT-PCR and immunoblotting, respectively. Additionally, proliferation and viability tests and time lapse-monitoring of movement of individual HBFs and IMR-90 cells were evaluated.
Results: We show that apigenin attenuates TGF-β_1-induced FMT in cultures of HBFs, and the magnitude of this attenuation was found to be similar to that observed in the established cell line of lung IMR-90 fibroblasts. Notably, FMT inhibition was observed at low (~10 μM), non-cytotoxic and non-cytostatic apigenin concentrations and could be correlated with the inhibition of α-SMA and tenascin C expression in HBFs at the mRNA level.
Conclusions: Our data are the first to demonstrate that apigenin inhibits the TGF-β_1-induced expansion of hyper-contractile, α-smooth muscle actin – positive myofibroblasts within populations of HBFs derived from asthmatic patients. They also indicate the possible interference of apigenin with bronchial wall remodeling during the asthmatic process *in vivo*.

Key words:
asthma, human bronchial fibroblasts, myofibroblasts, IMR-90 cells, transforming growth factor β, apigenin

Introduction

Bronchial asthma is one of the most common diseases throughout the world and its rate of prevalence has been continuously increasing over recent decades [2, 15, 41]. Pulmonary fibrosis, a basic hallmark of the progression of asthmatic respiratory disability, involves airway remodeling and is characterized by lo-

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cal epithelial injury, increased smooth muscle mass, extracellular matrix deposition and the accumulation of myofibroblasts mainly due to fibroblast-to-myofibroblast transition (FMT) [1, 12, 35, 47]. Bronchial wall remodeling develops in response to a range of external stimuli including signals from an inadequately activated immune system such as TGF-β. However, bronchial fibroblasts derived from asthmatic patients also have some inherent features which facilitate their differentiation into myofibroblasts, spreading into the wound matrix and developing contractile features [36, 37]. It is generally believed that the interplay between these extrinsic and intrinsic factors stimulates and/or facilitates phenotypic transitions in populations of lung fibroblasts thus determining personal susceptibility to pro-asthmatic cues [24]. Although the commonly used anti-asthmatic regimens, including steroid agents and leukotriene modifiers, mainly interfere with the pro-inflammatory activity of asthmatic inflammation [5, 6, 23, 46], their direct effect on the phenotypic shift leading to lung fibrosis remains disputable [8, 13, 34].

Recent epidemiological studies indicate that the application of dietary flavonoids for which anti-atherosclerotic and anti-carcinogenic activity has been suggested [29, 40] may also be considered as a complementary form of preventive anti-asthmatic strategy [28, 43]. In particular, apigenin (4',5,7-trihydroxyflavone) is a non-mutagenic flavone abundant in many herbs and vegetables, including chamomile, thyme, parsley and broccoli, and displays cytostatic and cytoprotective activity [10, 11, 33, 45]. The tentative anti-asthmatic activity of apigenin was deduced on the basis of interference with the pro-inflammatory responses of the immune system to allergens [17, 22, 32]. For instance, apigenin was demonstrated to compromise the secretion of a range of cytokines in activated basophiles [28]. However, its effect on asthmatic airway remodeling and especially on responsiveness of the cells constituting bronchial walls to pro-inflammatory cues was not thoroughly addressed.

In particular, it is unknown whether apigenin affects the sensitivity of human bronchial fibroblasts (HBFs) to the stimulating effect of TGF-β_1 on cell transition into the myofibroblastic phenotype. Fibroblasts propagated *in vitro* from the *ex vivo* bronchial biopsies of patients with diagnosed asthma are characterized by susceptibility to FMT in response to exogenous TGF-β [36, 37]. Therefore, we used this model to elucidate if apigenin interferes with FMT when administered at non-cytotoxic and physiologically relevant concentrations. These *in vitro* studies enabled a demonstration of the attenuating effect of apigenin on TGF-β_1-induced FMT in primary HBFs from asthmatic patients, comparable to that observed in the established cell line of lung embryonic myofibroblastic IMR-90 [42].

Materials and Methods

Cells and culture conditions

Human embryonic lung fibroblasts (IMR-90) were cultured in MEM with Earle Salts medium (PAA Laboratories GmbH, Austria) supplemented with 10 μM non-essential amino acids (PAA), 10 μM sodium pyruvate (PAA), 10% fetal bovine serum (FBS; Gibco, Carlsbad, CA, USA), and gentamycin (125 μg/ml, Krka d.d., Novo Mesto, Slovenia).

Primary human bronchial fibroblasts (HBFs) were isolated from bronchial biopsies obtained during bronchoscopy examination from 8 patients diagnosed with moderate asthma (AS), as described previously [36, 37]. All patients were treated in the Department of Medicine of the Jagiellonian University and were in stable clinical condition. The study was approved by the Jagiellonian University's Ethics Committee (KBET/362/B/2003) and informed, written consent was obtained from all study participants. HBFs were cultured in DMEM with 10% fetal bovine serum (FBS) at 37°C in a humidified atmosphere with 5% CO_2 and used between 5–15 passages. Human recombinant TGF-β_1 (BD Biosciences, Franklin Lakes, NJ, USA), 0.1% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO, USA) and apigenin (Sigma-Aldrich) were administered in various combinations to the fresh culture medium where indicated. Apigenin was diluted in the culture medium from freshly made stock solution (20 mg/ml in dimethyl sulfoxide, DMSO; Sigma-Aldrich) to the working concentrations (1, 5, 10 and 20 μM). Medium containing an amount of DMSO equivalent to the highest applied apigenin concentration (0.27‰) was taken as a solvent control. At this concentration, DMSO did not exert an effect on IMR-90 and HBF proliferation, viability or TGF-β_1-induced FMT (data not shown).

Proliferation and viability tests

For cell viability tests, cells were seeded onto 12-well plates at 2×10^4 density per well. 24 h after seeding, the cultured medium was replaced with the same medium supplemented with 10% FBS containing a range of apigenin concentrations (from 1 to 20 μM) or DMSO (equivalent to 20 μM apigenin). Cell viability was determined by the fluoresceine diacetate/ethidium bromide (EB) test using the Leitz Orthoplan fluorescence microscopy system and expressed as percent of fluoresceine-positive/EB-negative cells.

For proliferation tests, cells were seeded onto 12-well plates at an initial density of 5×10^3 cells/cm^2, incubated in DMEM with 10% FCS for 24 h at 37°C, and then the culture medium was replaced with a fresh one containing DMSO or apigenin. The incubation time varied from 1 to 5 days, next, the cells were harvested by trypsinization and counted using Bürker's chamber.

Time lapse-monitoring of movement of individual cells

Cell movement was observed with an inverted Leica microscope with IMC contrast optics, at 37°C and 5% CO_2 atmosphere. IMR-90 cells or HBFs were seeded into 6-well plates at a density of 2×10^2 cells/cm^2 and incubated in DMEM supplemented with 10% FBS and gentamycin for 24 h. Subsequently, the medium was replaced with fresh culture medium (control) or the same medium containing 10 μM apigenin and cell movement was recorded for 440 min at 5 min time intervals. The cell trajectories were presented in circular diagrams with the starting point of each trajectory situated at the plot center. The Hiro program written by W. Czapla was used for analysis of parameters characterizing cell locomotion as described previously [16]. For each data point measured, 50–400 cells were analyzed. Statistical significance was determined using a non-parametric Mann-Whitney U-test with $p < 0.05$ considered to indicate significant differences.

Immunofluorescence staining and FMT analyses

Myofibroblasts were identified by immunodetection of α-SMA as described previously [37]. In brief, IMR-90 cells and HBFs derived from asthmatic patients (AS) were cultured in DMEM with 10% FBS for 24 h, then with DMEM with 0.1% BSA (control) and in the same medium supplemented with TGF-β_1 (5 ng/ml) and DMSO (equivalent to 20 μM apigenin); or TGF-β_1 and apigenin (1–20 μM) for 7 days. Cells growing on glass coverslips were fixed in 3.7% paraformaldehyde, permeated in 0.1% Triton X-100, blocked with 1% BSA and incubated with mouse monoclonal antibody against human α-SMA (clone 1A4, Sigma-Aldrich), Alexa Fluor 488 goat anti-mouse IgG (clone A11001, Sigma-Aldrich) and counterstained with Hoechst 33342 (Sigma-Aldrich). Estimation of the percentage of α-SMA positive cells in specimens mounted in polyvinyl alcohol (Mowiol; Sigma-Aldrich) was performed with a Leica DM IRE2 microscope.

Quantitative real-time RT-PCR analysis

Real-time PCR was used to detect and quantify expression of α-SMA and tenascin C transcripts. Cells were seeded onto Petri dishes and incubated in DMEM with 10% FCS for 24 h, then with DMEM supplemented 0.1% BSA and TGF-β_1 (5 ng/ml) with or without apigenin (10 μM) for 24 h. Total RNA was extracted by chaotropic lysis using an RNA extraction kit (A&A Biotechnology, Gdynia, Poland) according to the manufacturer's protocol and cDNA was generated by reverse transcription using the High Capacity cDNA Reverse Transcriptase Kit (Life Technologies - Applied Biosystems, Foster City, CA, USA) in the presence of RNase inhibitor. Real-time PCR assays were done using iCycler Real Time PCR equipment (Biorad, Karlsbad, CA, USA) and Sybr Green (Amresco, Solon, OH, USA) detection of amplification products. Specific α-SMA primers (5′-AATGATTCATAGGGCTTCAG-3′, 5′-ATTTGACCCAGAACTACTTT-3′), tenascin C primers (5′-CCAGCGACCATCAACGCAGC-3′, 5′-GGGGCTTGTTCAGTGGATGCCT-3′) and housekeeping GAPDH (5′-AGAACATCATCCCTGCCTCTAC-3′,5′-CTGTTGAAGTCA GAGGAGACCA-3′) were used to quantify the transcripts in each cell line tested. The relative abundance of specific mRNA transcripts was estimated on the basis of a cycle threshold value (CT) and recalculated against the housekeeping gene using the ΔCT method. ΔCT refers to $CT_{GAPDH} - CT_{\alpha\text{-SMA}}$ or $CT_{TENASCIN\ c}$. All samples were run in triplicate (n = 3). Primer design and control gel electrophoresis of amplification products ensured artifact-free results.

Preparation of protein extracts and immunoblotting

Cells were cultured in Petri dishes in DMEM with 10% FCS for 24 h, then with DMEM supplemented 0.1% BSA (control conditions) or stimulated with TGF-β_1 (5 ng/ml) and apigenin (10 μM) until confluence for 7 days. Afterwards, total cell proteins were collected using the lysis buffer (0.1 mol/l Tris-HC1, 15% glycerol, 2 mM EDTA, 2% SDS, 10 mM phenylmethylsulfonyl fluoride, and 10 μg/ml aprotinin, pH 7.4). The protein concentration was determined using the Bradford method and aliquots containing 10 μg of protein from whole cell lysates were separated by 15% SDS-polyacrylamide gel electrophoresis under reducing conditions and then transferred onto a polyvinylidene difluoride membrane (Hybond-P, Amersham Pharmacia Biotech, Buckinghamshire, England). After blocking in PBS-T (0.1% Tween 80 in PBS) containing 5% skimmed milk at room temperature, the membrane was incubated with mouse monoclonal anti-α-SMA antibody (Sigma-Aldrich, 1 : 1000) and with mouse monoclonal anti-GAPDH antibody (Sigma-Aldrich, 1 : 10000). After washing, the membrane was incubated with anti-mouse IgG horseradish peroxidase-conjugated antibody (1 : 3000, Invitrogen, Carlsbad, CA, USA). The extensively washed membranes were incubated with the chemiluminescent reagent Super Signal West Pico Substrate (Pierce, Rockford, IL, USA) and exposed to Kodak X-Omat film. ROD (relative optical density) was estimated using ImageJ 1.45s (NIH) software. α-SMA protein levels were quantified by densitometry and normalized to the expression of GAPDH.

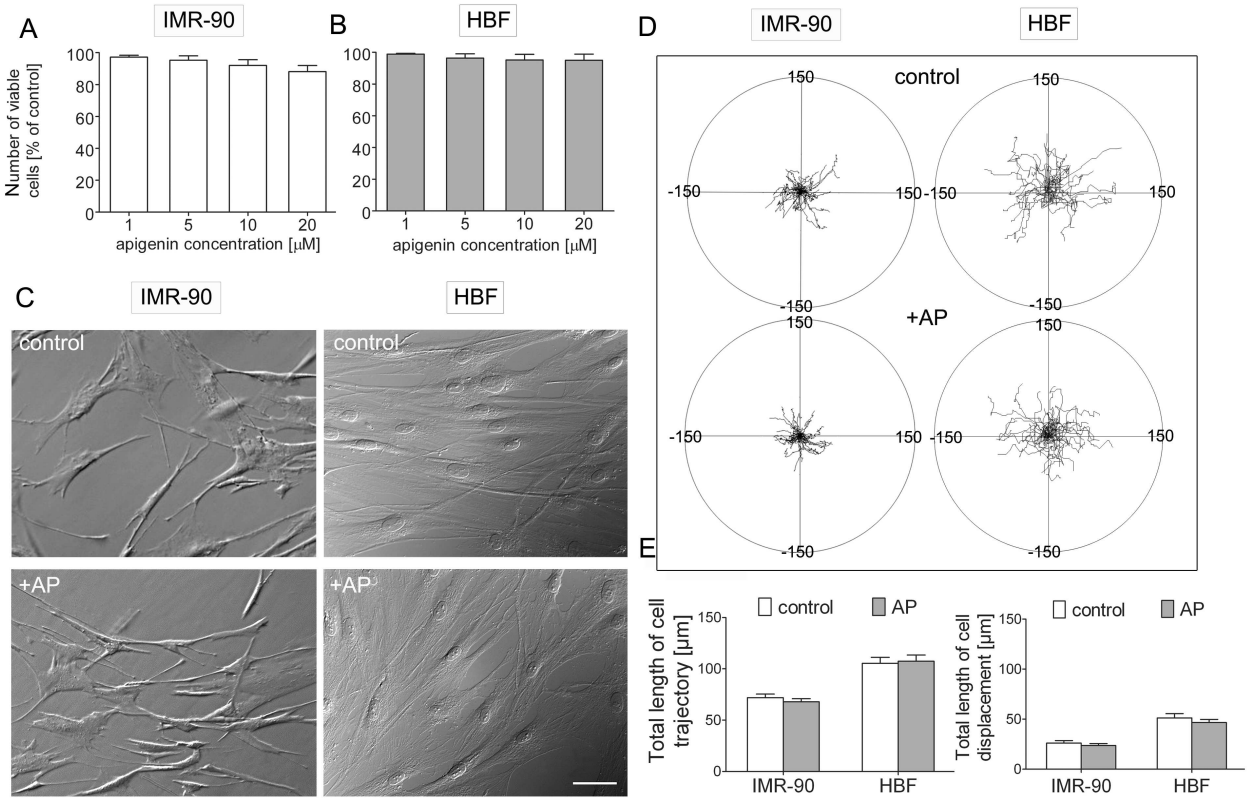

Fig. 1. Effect of apigenin on the viability (**A**, **B**), morphology (**C**) and motility (**D**, **E**) of lung fibroblasts. Apigenin (0–20 μM) present in culture medium supplemented with 10% FBS did not affect the relative number of viable IMR-90 cells (**A**) and HBFs derived from asthmatic patients (**B**) after 7-day incubation as demonstrated by the fluorescein diacetate/ethidium bromide viability tests (**A**, **B**) and representative images of the morphology of IMR-90 cells and HBFs cultured in the presence of 10 μM apigenin (AP; **C**). Likewise, the influence of apigenin (AP, 10 μM) on the movement of IMR-90 cells and HBFs could not be detected by time-lapse analyses of their trajectories (**D**) and quantitative analyses of cell movement parameters: total length of cell trajectory (μm) and total length for displacement (μm; **E**). Cell trajectories are presented in the form of a circular diagram (axis scale in μm) drawn with the initial point of each trajectory placed at the origin of the plot. Values represent the mean ± SD; results are representative of three independent experiments. The lack of significant differences between control and apigenin stimulated fibroblasts was determined by analysis of variance (**A**, **B**) and a non-parametric Mann-Whitney test (**E**) * $p < 0.05$. Bar = 50 μm

Results

Apigenin does not affect the viability of lung fibroblasts when applied at physiologically relevant concentrations

Apigenin was previously demonstrated to induce apoptotic and necrotic responses in versatile cellular systems [44]. Because remnants of necrotic cells may evoke the inflammatory response *in vivo*, viability tests were first performed in order to identify non-cytotoxic concentrations of apigenin. As illustrated in Figure 1A, a minute cytotoxic effect was demonstrated only by IMR-90 cells that were exposed to 20 μM apigenin for 3 to 5 days. The impact of apigenin on the viability of primary bronchial fibroblasts derived from patients with asthma was even less pronounced and statistically non-significant (Fig. 1B). These data indicate that apigenin, when administered at concentrations up to 10 μM, does not affect cell viability, which could bias our further observations. The lack of deleterious effects of apigenin was further verified by time-lapse and morphometric analyses which did not reveal an effect of 10 μM apigenin on the morphology (Fig. 1C) or motility (Fig. 1D) of IMR-90 cells and HBFs.

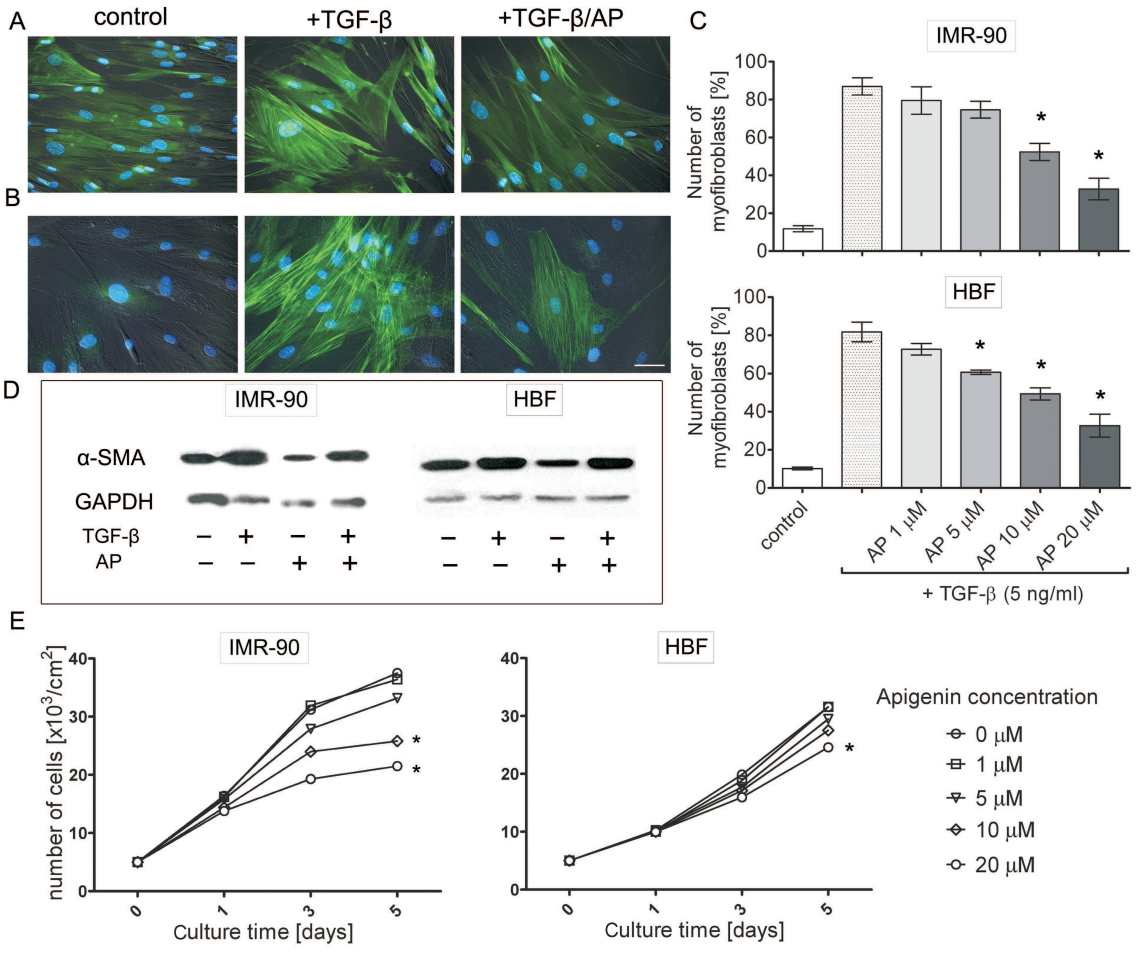

Fig. 2. Effect of apigenin on TGF-β_1- stimulated FMT in IMR-90 cells and HBF populations. IMR-90 cells and HBFs derived from asthmatic patients (characterized by high susceptibility to TGF-β_1-induced FMT) were cultured in DMEM supplemented with TGF-β_1 (5 ng/ml), or TGF-β_1 and apigenin (1–20 μM) for 7 days, and immunostained for α-SMA (green) and DNA (blue). Ten μM apigenin attenuated TGF-β_1-stimulated FMT in IMR-90 (**A**) and asthmatic HBF populations (**B**) in a dose-dependent manner as demonstrated by decreasing numbers of α-SMA – positive cells in the presence of increasing apigenin concentrations (**C**) and representative immunoblots showing decreased α-SMA protein levels in apigenin-treated cell populations (**D**). This effect was paralleled by a distinct inhibition of cell proliferation in IMR-90, but not HBF populations (**E**). Bars represent the mean values (± SD) of at least three separate experiments. Statistical significance of differences between cells stimulated only with TGF-β_1 and TGF-β_1 with apigenin was determined by analysis of variance, * $p < 0.05$. Scale bar = 50 μm

Apigenin attenuates TGF-β_1-induced FMT in human lung fibroblast populations in a dose-dependent manner

Since TGF-β has a central role in the inflammatory processes of the bronchial wall leading to airway remodeling in asthma, and apigenin was recognized as a bioactive flavonoid possessing anti-inflammatory properties [1], we further focused on the interrelations between the cytotoxic and cytostatic effects and the interference of apigenin with TGF-β_1-induced FMT in HBFs derived from asthmatic patients. For this purpose, the sensitivity of HBFs and IMR-90 cells to apigenin-induced attenuation of FMT was compared. Increased α-SMA positive cell fraction observed upon TGF-β_1 treatment confirmed that TGF-β_1 triggered FMT in analyzed cell populations. Apigenin decreased the magnitude of FMT in IMR-90 (Fig. 2A) and HBF populations (Fig. 2B) as illustrated by a dose-dependent reduction of α-SMA-positive cell fractions (Fig. 2C). A considerable effect was observed in HBF populations in the presence of 5 μM apigenin, whereas 10 μM apigenin significantly inhibited FMT in both cell populations. This observation was further confirmed by reduced levels of α-SMA expression in TGF-β_1/10 μM apigenin-treated IMR-90 cells and HBFs (Fig. 2D). Surprisingly, a less prominent dose-dependent effect of apigenin on the proliferation of HBFs than on IMR-90 cells was observed across all concentrations tested (Fig. 2E). Thus, apigenin affected TGF-β_1-induced FMT in HBF populations independently of its cytostatic and cytoxic activity.

Apigenin interferes with FMT in HBF populations derived from asthmatic patients

We have shown previously that the cellular model based on primary HBFs derived from asthmatic patients enables comparative analyses of their sensitivity to extrinsic stimuli [37, 38]. Further experiments were performed to evaluate the effect of apigenin on FMT in HBF populations derived from asthmatic patients (n = 5) characterized by differing reactivity to TGF-β_1 (from 65% to 86% of myofibroblasts in HBF populations after 7 day culture in medium containing 5 ng/ml TGF-β_1; data not shown). We analyzed FMT-related HBF responses in the presence of 10 μM apigenin because at this concentration apigenin significantly inhibited TGF-β_1-induced FMT in IMR-90 and HBF populations (Fig. 2). Fibroblasts from all analyzed HBF cultures reacted to apigenin by exhibiting considerable FMT inhibition, however, the extent of apigenin-mediated FMT attenuation varied from 20 to 63% (Fig. 3A) which corresponded to EC_{50} values ranging between 2.25 and 8.84 μM. Parallel immunoblot (Fig. 3B) and quantitative PCR analyses (Fig. 3C) revealed attenuating effects of 10 μM apigenin on α-SMA expression at the protein and mRNA

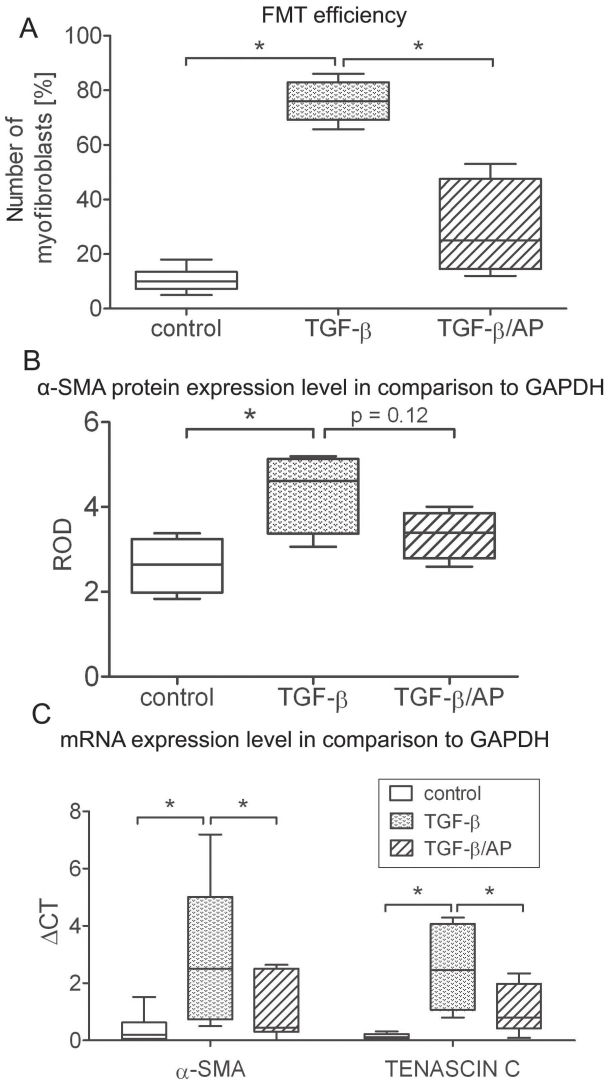

Fig. 3. Effect of 10 μM apigenin on FMT efficiency and the expression of FMT markers in HBF populations derived from asthmatic patients. Apigenin attenuated, albeit to a different extent, FMT in all tested HBF populations (n = 5) as demonstrated by the relative numbers of α-SMA – positive cells (**A**), densitometrically quantified α-SMA protein levels (**B**), and α-SMA and tenascin C mRNA levels quantified with real-time PCR (**C**). Bars on graphs represent the mean values (the means of at least three separate experiments for each culture). The significance of differences between cells stimulated with 5 ng/ml TGF-β_1 alone and TGF-β_1 with apigenin was determined by analysis of variance, * $p < 0.05$

levels. In addition, apigenin significantly reduced the expression of tenascin C (another marker of myofibroblasts and the asthmatic process in bronchial airways [7]) at the mRNA level in all analyzed HBF populations. These observations support the biological relevance of the attenuating effect of apigenin on TGF-β_1-induced FMT in HBF populations and suggest the possible anti-asthmatic activity of apigenin *in vivo*.

Discussion

A number of epidemiological studies revealed that the dietary uptake of flavonoids in general, and apigenin in particular, is negatively correlated with incidence of allergic and inflammatory syndromes including asthma [2, 3, 39, 43]. However, the mechanisms involved remain largely obscure. In particular, little was known about the molecular interference of apigenin with processes leading to bronchial wall remodeling in asthma, such as TGF-β-induced FMT. This is partly due to methodological constraints since *in vivo* studies on animals and clinical samples can only provide a snapshot of the fibrotic cascade at the point of tissue harvest. The major limitation of *in vitro* models based on embryonal myofibroblastic cell lines is that they provide casuistic data prone to bias due to epigenetic modifications occurring during prolonged cell expansion *in vitro*. Analyses of FMT dynamics within primary HBFs expanded directly from bronchial biopsies of patients with asthma used in this study provide a more natural approach. Moreover, a quantitative comparison of the sensitivity of bronchial fibroblasts sampled from different patients to apigenin in fully controlled experimental conditions is possible. We have recently shown that these cells retained inherent features facilitating their FMT in response to external stimuli, such as TGF-β [37, 38]. Here, we demonstrate that apigenin attenuates TGF-β_1-induced FMT in populations of HBFs derived from asthmatic patients at a rate comparable to the effect on embryonal myofibroblastic IMR-90 cells.

Induction of FMT in HBFs and IMR-90 cells by exogenous TGF-β_1 mimics the *in vivo* asthmatic process triggered by local secretion of TGF-β within the inflammatory milieu. Our observations suggest that at the single-cell level, apigenin reduces the probability of HBF entry into the FMT program. Thus, these data add TGF-β_1-signaling in HBFs derived from asthmatic patients to the long list of apigenin targets and indicate that this flavonoid may attenuate *in vivo* airway wall remodeling *via* a TGF-β desensitizing effect on the HBFs. Importantly, attenuation of TGF-β_1-induced FMT was observed in all analyzed HBF populations in the presence of low (~10 μM), non-cytotoxic and non-cytostatic apigenin concentrations, corresponding to those expected for flavonoids in sera of patients after prolonged dietary uptake of these compounds [28, 49]. This observation demonstrates that the attenuating effect of apigenin on phenotypic transitions in the analyzed cell populations is independent of its cytotoxic activity. Altogether, we demonstrate for the first time that apigenin may affect TGF-β_1-induced FMT without exerting side-effects on other properties of HBFs. This is important since HBFs are crucial for the maintenance of bronchial wall homeostasis [35, 48] and their apoptosis and/or necrosis might result in inadequate responses of the immune system, instigating secondary inflammation.

Apigenin was previously suggested to attenuate fibrotic changes in other tissues (kidney, liver) *via* direct inhibitory effects on the function of the immune system and/or inhibition of production and secretion of pro-inflammatory cytokines such as TNF-α [30] and TGF-β_1 [18]. However, our data indicate that apigenin directly interferes with the activity of the TGF-β-dependent signaling pathway. This is not surprising since this flavonoid displays considerable pleiotropy. It targets the activity of a number of secondary messengers, including those potentially involved in cell reactions to TGF-β_1, such as NF-κB [27], PKC [4], FAK [14, 25], MAPK/ERK [26] and PI3K-Akt [45] in a cell context-dependent manner. Apigenin was shown to suppress PDGF-induced vascular smooth muscle cell activation through the blockade of downstream signal transduction, including STAT, ERK1/2 and Akt [19]. Importantly, the relevance of the Akt-dependent pathway for the reactions of HBFs described in this study is supported by the data of Ricupero et al. [42], who demonstrated that the inhibition of Akt signaling by apigenin, but not MAP and SMAD2-dependent pathways, predominantly accounts for its inhibitory effect on the expression of myofibroblastic markers (α-SMA and type I collagen) in populations of IMR-90 cells exposed to TGF-β.

Although further research is necessary to identify the mechanisms of HBF reactivity to apigenin, our

observations suggest therapeutic benefits of apigenin in the prevention of bronchial asthma remodeling. Specifically, we demonstrate that apigenin can interfere with the events directly involved in fibrotic changes. Because other flavonoids such as luteolin were found to affect fibrotic processes in a similar way to apigenin [9], these findings may facilitate the development of new therapeutic approaches based on plant polyphenolic compounds aimed at the prevention of bronchial wall remodeling during asthma progression.

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References:

1. Al-Muhsen S, Johnson JR, Hamid Q: Remodeling in asthma. J Allergy Clin Immunol, 2011, 128, 451–462.
2. Anandan C, Nurmatov U, van Schayck OC, Sheikh A: Is the prevalence of asthma declining? Systematic review of epidemiological studies. Allergy, 2010, 65, 152–167.
3. Arts IC, Hollman PC: Polyphenols and disease risk in epidemiologic studies. Am J Clin Nutr, 2005, 81, 317–325.
4. Balasubramanian S, Zhu L, Eckert RL: Apigenin inhibition of involucrin gene expression is associated with a specific reduction in phosphorylation of protein kinase Cδ Tyr^{311}. J Biol Chem, 2006, 281, 36162–36172.
5. Barnes PJ: Corticosteroids: the drugs to beat. Eur J Pharmacol, 2006, 8, 2–14.
6. Bosnjak B, Stelzmueller B, Erb KJ, Epstein MM: Treatment of allergic asthma: modulation of Th2 cells and their responses. Respir Res, 2011, 25, 102–114.
7. Brellier F, Tucker RP, Chiquet-Ehrismann R: Tenascins and their implications in diseases and tissue mechanics. Scand J Med Sci Sports, 2009, 19, 511–519.
8. Chakir J, Shannon J, Molet S, Fukakusa M, Elias J, Laviolette M, Boulet LP, Hamid Q: Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on TGF-β, IL-11, IL-17, and type I and type III collagen expression. J Allergy Clin Immunol, 2003, 111, 1293–1298.
9. Chen CY, Peng WH, Wu LC, Wu CC, Hsu SL: Luteolin ameliorates experimental lung fibrosis both in vivo and in vitro: implications for therapy of lung fibrosis. J Agric Food Chem, 2010, 24, 11653–11661.
10. Clere N, Faure S, Martinez MC, Andriantsitohaina R: Anticancer properties of flavonoids: roles in various stages of carcinogenesis. Cardiovasc Hematol Agents Med Chem, 2011, 1, 62–77.
11. Czyż J, Madeja Z, Irmer U, Korohoda W, Hülser DF: Flavonoid apigenin inhibits motility and invasiveness of carcinoma cells in vitro. Int J Cancer, 2005, 10, 12–18.
12. Descalzi D, Folli C, Scordamaglia F, Riccio AM, Gamalero C, Canonica GW: Importance of fibroblasts-myofibroblasts in asthma-induced airway remodeling. Recent Pat Inflamm Allergy Drug Discov, 2007, 1, 237–241.
13. Durrani SR, Viswanathan RK, Busse WW: What effect does asthma treatment have on airway remodeling? Current perspectives. J Allergy Clin Immunol, 2011, 128, 439–448.
14. Franzen CA, Amargo E, Todorović V, Desai BV, Huda S, Mirzoeva S, Chiu K et al.: The chemopreventive bioflavonoid apigenin inhibits prostate cancer cell motility through the focal adhesion kinase/Src signaling mechanism. Cancer Prev Res (Phila), 2009, 9, 830–841.
15. Gaffin JM, Phipatanakul W: The role of indoor allergens in the development of asthma. Curr Opin Allergy Clin Immunol, 2009, 9, 128–35.
16. Galanty A, Michalik M, Sędek L, Podolak I: The influence of LTS-4, a saponoside from *Lysimachia thyrsiflora* L., on human skin fibroblasts and human melanoma cells. Cell Mol Biol Lett, 2008, 13, 585–598.
17. González R, Ballester I, López-Posadas R, Suárez MD, Zarzuelo A, Martínez-Augustin O, Sánchez de Medina F: Effects of flavonoids and other polyphenols on inflammation. Crit Rev Food Sci Nutr, 2011, 51, 331–362.
18. Grewal JS, Mukhin YV, Garnovskaya MN, Raymond JR, Greene EL: Serotonin 5-HT_{2A} receptor induces TGF-β1 expression in mesangial cells via ERK: proliferative and fibrotic signals. Am J Physiol, 1999, 276, F922–930.
19. Guan H, Gao L, Zhu L, Yan L, Fu M, Chen C, Dong X et al.: Apigenin attenuates neointima formation via suppression of vascular smooth muscle cell phenotypic transformation. J Cell Biochem, 2012 ,113, 1198–1207.
20. Halwani R, Al-Muhsen S, Al-Jahdali H, Hamid Q: Role of transforming growth factor-β in airway remodeling in asthma. Am J Respir Cell Mol Biol, 2011, 44, 127–133.
21. Hardie WD, Glasser SW, Hagood JS: Emerging concepts in the pathogenesis of lung fibrosis. Am J Pathol, 2009, 175, 3–16.
22. Hirano T, Higa S, Arimitsu J, Naka T, Shima Y, Ohshima S, Fujimoto M et al.: Flavonoids such as luteolin, fisetin and apigenin are inhibitors of interleukin-4 and interleukin-13 production by activated human basophils. Int Arch Allergy Immunol, 2004, 134, 135–140.
23. Holgate ST: A look at the pathogenesis of asthma: the need for a change in direction. Discov Med, 2010, 9, 439–447.
24. Holgate ST: Genetic and environmental interaction in allergy and asthma. J Allergy Clin Immunol, 1999, 104, 1139–1146.
25. Hu XW, Meng D, Fang J: Apigenin inhibited migration and invasion of human ovarian cancer A2780 cells

through focal adhesion kinase. Carcinogenesis, 2008, 29, 2369–2376.
26. Hwang YP, Oh KN, Yun HJ, Jeong HG: The flavonoids apigenin and luteolin suppress ultraviolet A-induced matrix metalloproteinase-1 expression via MAPKs and AP-1-dependent signaling in HaCaT cells. J Dermatol Sci, 2011, 61, 23–31.
27. Kang OH, Lee JH, Kwon DY: Apigenin inhibits release of inflammatory mediators by blocking the NF-κB activation pathways in the HMC-1 cells. Immunopharmacol Immunotoxicol, 2011, 33, 473–479.
28. Kawai M, Hirano T, Higa S, Arimitsu J, Maruta M, Kuwahara Y, Ohkawara T et al.: Flavonoids and related compounds as anti-allergic substances. Allergol Int, 2007, 56, 113–123.
29. Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD: Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med, 2002, 113, Suppl 9B, 71S–88S.
30. Kumazawa Y, Kawaguchi K, Takimoto H: Immunomodulating effects of flavonoids on acute and chronic inflammatory responses caused by tumor necrosis factor alpha. Curr Pharm Des, 2006, 12, 4271–4279.
31. Lepley DM, Pelling JC, Lepley DM, Pelling JC: Induction of p21/WAF1 and G1 cell-cycle arrest by the chemopreventive agent apigenin. Mol Carcinog, 1997, 19, 74–82.
32. Li RR, Pang LL, Du Q, Shi Y, Dai WJ, Yin KS: Apigenin inhibits allergen-induced airway inflammation and switches immune response in a murine model of asthma. Immunopharmacol Immunotoxicol, 2010, 32, 364–370.
33. Makinde T, Murphy RF, Agrawal DK: The regulatory role of TGF-beta in airway remodeling in asthma. Immunol Cell Biol, 2007, 85, 348–356.
34. Mauad T, Bel EH, Sterk PJ: Asthma therapy and airway remodeling. J Allergy Clin Immunol, 2007, 120, 997–1009.
35. McAnulty RJ: Fibroblasts and myofibroblasts: their source, function and role in disease. Int J Biochem Cell Biol, 2007, 39, 666–671.
36. Michalik M, Pierzchalska M, Legutko A, Ura M, Ostaszewska A, Soja J, Sanak M: Asthmatic bronchial fibroblasts demonstrate enhanced potential to differentiate into myofibroblasts in culture. Med Sci Monit, 2009, 15, 194–201.
37. Michalik M, Pierzchalska M, Włodarczyk A, Wójcik KA, Czyż J, Sanak M, Madeja Z: Transition of asthmatic bronchial fibroblasts to myofibroblasts is inhibited by cell-cell contacts. Respir Med, 2011, 105, 1467–1475.
38. Michalik M, Wójcik KA, Jakieła B, Szpak K, Pierzchalska M, Sanak M, Madeja Z, Czyż J: Lithium attenuates TGF-β1-induced fibroblasts to myofibroblasts transition in bronchial fibroblasts derived from asthmatic patients. J Allergy, 2012, 2012:206109, doi: 10.1155/2012/206109.
39. Nagel G, Weinmayr G, Kleiner A, Garcia-Marcos L, Strachan DP: ISAAC Phase Two Study Group: Effect of diet on asthma and allergic sensitisation in the International Study on Allergies and Asthma in Childhood (ISAAC) Phase Two. Thorax, 2010, 65, 516–522.
40. Pauwels EK: The protective effect of the Mediterranean diet: focus on cancer and cardiovascular risk. Med Princ Pract, 2011, 20, 103–111.
41. Peters SP, Ferguson G, Deniz Y, Reisner C: Uncontrolled asthma: a review of the prevalence, disease burden and options for treatment. Respir Med, 2006, 100, 1139–1151.
42. Ricupero DA, Poliks CF, Rishikof DC, Kuang PP, Goldstein RH: Apigenin decreases expression of the myofibroblast phenotype. FEBS Lett, 2001, 28, 15–21.
43. Shaheen SO, Sterne JA, Thompson RL, Songhurst CE, Margetts BM, Burney PG: Dietary antioxidants and asthma in adults: population-based case-control study. Am J Respir Crit Care Med, 2001, 164, 1823–1828.
44. Shukla S, Gupta S: Apigenin: a promising molecule for cancer prevention. Pharm Res, 2010, 27, 962–978.
45. Shukla S, Gupta S: Apigenin-induced cell cycle arrest is mediated by modulation of MAPK, PI3K-Akt, and loss of cyclin D1 associated retinoblastoma dephosphorylation in human prostate cancer cells. Cell Cycle, 2007, 2, 1102–1114.
46. Strzępa A, Szczepanik M: IL-17-expressing cells as a potential therapeutic target for treatment of immunological disorders. Pharmacol Rep, 2011, 63, 30–44.
47. Westergren-Thorsson G, Larsen K, Nihlberg K, Andersson-Sjöland A, Hallgren O, Marko-Varga G, Bjermer L: Pathological airway remodelling in inflammation. Clin Respir J, 2010, 4, 1–8.
48. Wilson MS, Wynn TA: Pulmonary fibrosis: pathogenesis, etiology and regulation. Mucosal Immunol, 2009, 2, 103–121.
49. Xiao ZP, Peng ZY, Peng MJ, Yan WB, Ouyang YZ, Zhu HL: Flavonoids health benefits and their molecular mechanism. Mini Rev Med Chem, 2011, 11, 169–177.

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