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Review

# Transcription factor Fra-2 and its emerging role in matrix deposition, proliferation and inflammation in chronic lung diseases

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ABSTRACT

Fos-related antigen-2 (Fra-2) belongs to the activator protein 1 (AP-1) family of transcription factors and is involved in a broad variety of cellular processes, such as proliferation or differentiation. Aberrant expression of Fra-2 or regulation can lead to severe growth defects or diverse pathologies. Elevated Fra-2 expression has been described in several chronic lung diseases, such as pulmonary fibrosis, chronic obstructive pulmonary disease and asthma. However, the pathomechanisms behind the Fra-2-induced pulmonary remodelling are still not fully elucidated.

Fra-2 overexpressing mice were initially described as a model of systemic sclerosis associated organ fibrosis, with predominant alterations in the lung. High levels of Fra-2 expression give rise to profound inflammation with severe remodelling of the parenchyma and the vasculature, resulting in fibrosis and pulmonary hypertension, respectively, but also alters bronchial function. In this review we discuss the central role of Fra-2 connecting inflammation, cellular proliferation and extracellular matrix deposition underlying chronic lung diseases and what we can learn for future therapeutic options.

## 1. Introduction

The activator protein (AP)-1 transcription factor belongs to the family of immediate early genes that are transcribed in a cell within minutes as a response to external or internal stimuli. Transcription factors orchestrate cellular responses to stimuli and regulate cellular behaviour such as proliferation or differentiation [1]. Many protein products of immediate early genes are transcription factors such as AP-1, NFkB, EGR-1 or CREB, which have important roles in the long-term regulation and control of cellular gene expression patterns. AP-1 complexes are not only master regulators of cellular behaviour but their aberrant regulation or expression can also lead to growth defects and disease development [1–4]. In particular, the lesser-studied AP-1 family member, Fra-2 has been shown to influence chronic lung disease development such as systemic sclerosis and pulmonary fibrosis [5]. However, the pathomechanisms underlying its role in chronic lung disease are poorly understood. Hence, the current review aims to elucidate and summarize the role of Fra-2 in in chronic lung diseases.

### 1.1. Fra-2 structure

The AP-1 family of transcription factors consists of Jun (c-jun, junB and junD) and Fos (c-fos, Fra-1, Fra-2 and fosB) proteins [6]. AP-1 was first identified to be activated by the phorbolester TPA (12-O-tetradecanoylphorbol-13-acetate), therefore the DNA sequence bound by AP-1 is called TPA-responsive element (TRE) and has the general consensus sequence TGAG/CTCA [7]. AP-1 belongs to the basic-region leucine zipper (bZIP) class of eukaryotic transcription factors that forms dimers via a leucine zipper domain (LZD) and bind to the palindromic TRE site through the adjacent basic amino acid rich domain [8]. While Jun proteins can exist as both homo- and heterodimers [9], Fos proteins cannot homo-dimerize and only form heterodimers with a Jun component [8]. In addition to the DNA binding domain, most of the AP-1 transcription factors have a transactivation domain (TAD), which is crucial for activation of the transcription of downstream target genes [8]. However, some members of the Fos family, including Fra-2, do not and are therefore dependent on the interaction with a TAD-containing dimerization partner [10]. Consequently, the DNA binding specificity and transactivation properties of Fra-2 are defined by the composition of the AP-1 dimer [11], which ultimately defines its target genes and their regulation.

Fra-2 is the most recently discovered and least described member of the AP-1 family. The human *FRA-2* gene was cloned from a cDNA library in the early nineties [12]. At the same time, Fra-2 was isolated from chicken embryo fibroblasts and identified as a 46 kDa protein with strong homology to FosB and Fra-1 [13]. Soon after, murine Fra-2 was isolated and characterized in more detail [14]. Due to this homology

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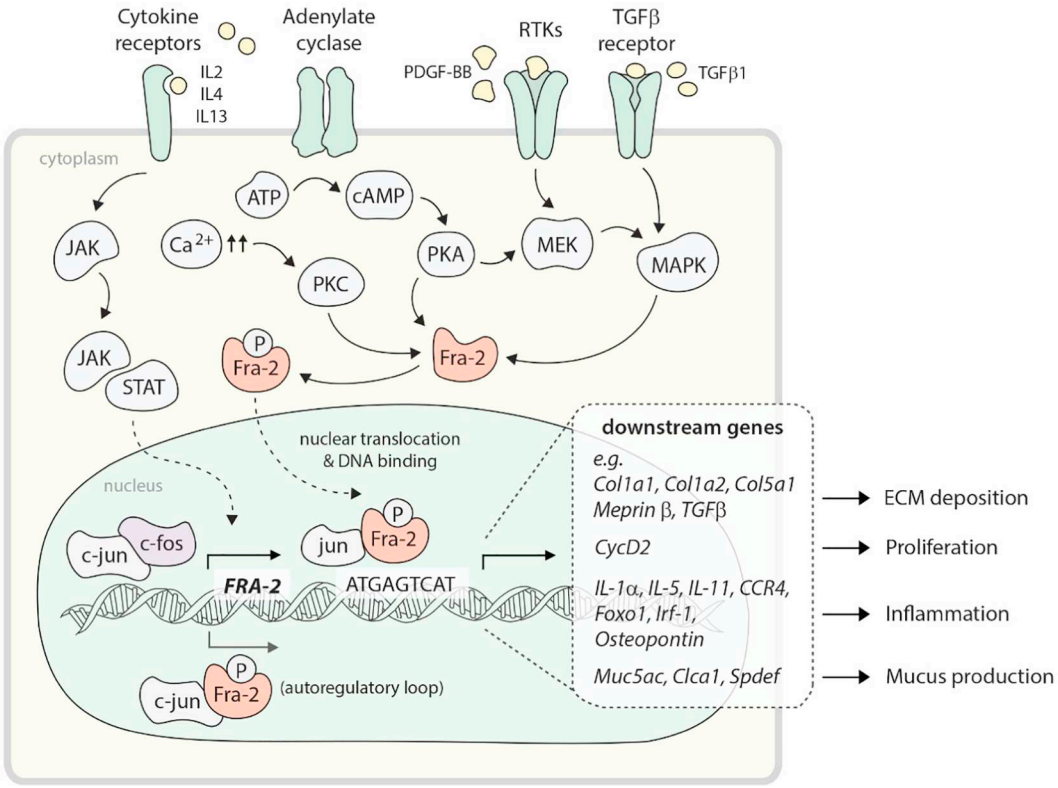


**Fig. 1.** Fra-2 regulation and downstream target genes.
The expression, post-translational modification and activity of Fra-2 can be controlled by multiple upstream stimuli, e.g. growth factors or inflammatory mediators lead to MAP kinase activation and Fra-2 phosphorylation, and thereby influence the transcriptional regulation of downstream genes. As newly formed Fra-2/c-jun dimers have lower transcriptional activity compared to c-fos/c-jun dimers, the newly transcribed Fra-2 can limit its own expression in a negative feedback loop. Other Fra-2 dimers bind to AP-1 consensus sites and induce transcription of target genes involved in a plethora of several processes, such as proliferation/apoptosis, extracellular matrix (ECM) deposition, inflammation and mucus production. MAPK: Mitogen Activated Protein Kinase; PKA: Protein Kinase A; PKC: Protein Kinase C; RTK: Receptor Tyrosine Kinase; PDGF-BB: Platelet Derived Growth Factor BB; TGFβ: Transforming Growth Factor beta; MEK: Mitogen Activated Protein Kinase Kinase; JAK: Janus Kinase; STAT: Signal Transducer and Activator of Transcription.

and its identification via an anti-fos antiserum, it was termed fos-related antigen 2 (Fosl2, Fra-2) [13]. The *Fra-2* gene has five regions homologous to other Fos proteins, including the basic leucine-zipper motif responsible for dimerization, but it differs from c-fos in lacking the strong TAD [8]. In line with this, c-Jun/Fra-2 dimers have a lower transactivation activity compared to c-Jun/c-Fos, however dimerization of Fra-2 with JunD increases the transactivation activity compared to JunD homodimers [15]. This differential transactivation activity of Fra-2 depending on its diverse dimerization partners makes Fra-2 an interesting but complex molecule to study.

### 1.2. Regulation of Fra-2

Regulation of AP-1 occurs on multiple levels. While AP-1 expression is regulated both transcriptionally and translationally, the activity and stability of AP-1 proteins is further influenced by post-translational modifications such as phosphorylation. Several stimuli can cause transcriptional upregulation of Fra-2 (Fig. 1), including phorbol esters (e.g. TPA), increased cAMP and $Ca^{2+}$ levels, serum [16] and diverse growth factors such as PDGF-BB or TGFβ [17,18]. Although expression of several Fos proteins can be regulated by the same stimuli, the kinetics of the response is different: While c-fos and FosB are very rapidly induced in response to serum and become undetectable again after 3 h, Fra-1 and Fra-2 expression is delayed, but remains elevated for longer [19]. Similar prolonged Fra-2 expression was observed upon induction with TPA, cAMP or $Ca^{2+}$ [16]. Furthermore, Fra-1 and Fra-2 are more stable compared to c-Fos and FosB which might account for their predominance in asynchronously growing cells [20].

The presence of an AP-1 binding site in the promoter region of Fra-2 also suggests auto-regulatory mechanisms and cross regulation between different AP-1 subunits [16,21]. Indeed, Fra-2 expression can be induced by c-jun/c-fos binding. Once Fra-2 translation is activated, Fra-2 becomes highly abundant and the c-jun/c-fos dimers are replaced by a c-jun/Fra-2 complex, which is reported to have lower transcriptional activity compared to c-fos/c-jun [15,21], suggesting an auto-regulatory negative-feedback loop of Fra-2 expression. Fra-2 expression can also be influenced by epigenetic modification changes, such as trimethylation of histone 3 (H3), which inhibits Fra-2 expression, whereas a lack of H3 trimethylation leads to aberrant Fra-2 expression [22].

Like other AP-1 components Fra-2 is not only regulated transcriptionally but can also be rapidly phosphorylated and activated by various stimuli leading to increased DNA binding [13]. For example, Fra-2 activity is influenced by TGFβ [23,24] and PDGF-BB [18], or by pro-inflammatory cytokines such as IL-13 [25]. In vitro Fra-2 is phosphorylated by several kinases, including cAMP-dependent kinase (PKA), protein kinase C (PKC), cyclin-dependent kinase 1-cdc2 (cdc2) and mitogen activated protein (MAP) kinases, however only MAP kinases induce phosphorylation of Fra-2 to a similar extent and in a similar pattern as observed in vivo [26]. Phosphorylation has a dual role on AP-1 transcription factors. On one hand, phosphorylation of Fra-2 by MAP kinases increases its DNA binding activity [26,27] and inhibition of the MAP kinase ERK blocks nuclear translocation and consequently Fra-2 DNA binding activity [18]. On the other hand, phosphorylation can also influence AP-1 protein stability and serve as a signal for degradation by the ubiquitin/proteasome system [28,29]. It has been indeed shown that JNK mediated phosphorylation on Ser73 of c-jun,

**Table 1**
Known direct target genes of Fra-2.

| Gene (Full name) | AP-1 binding partner | Regulation | Details | Reference |
|---|---|---|---|---|
| *Fra1/Fosl1 (fos-related antigen 1)* | cJun | Enhanced | In human A549 alveolar epithelial cells, validated by ChIP | Adiseshaiah et al. [32] |
| *CCND1* (cyclin D1) | cJun | Enhanced | In vitro, validated by luciferase reporter assay and EMSA | Bakiri et al. [33] |
| *CCNA2* (cyclin A2) | cJun | Enhanced | In vitro, validated by luciferase reporter assay and EMSA | Bakiri et al. [33] |
| *IL1A* (interleukin-1α) | Unknown | Enhanced | In human lung parenchymal fibroblast; validated by EMSA | Birnhuber et al. [34] |
| *Adipoq* (adiponectin, C1Q and collagen domain containing) | cJun/JunD | Repressed | In mouse osteoblasts, validated by ChIP | Bozec et al. [35] |
| *Bglap2* (bone gamma-carboxyglutamat protein/osteocalcin) | unknown | Enhanced | Mouse osteoblasst with deletion or activation of Fra-2, in vitro osteocalcin production | Bozec et al. [35] |
| *Col1a2* (collagen type 1 α 2 chain) | cJun/JunB | Enhanced | In mouse osteoblasts, validated by ChIP and luciferase reporter assay | Bozec et al. [36] |
| *Lif* (LIF interleukin-6 family cytokine/leukemia inhibitory factor) | cJun | Enhanced | In bone tissue, validated by ChIP and luciferase reporter assay | Bozec et al. [37] |
| *Rgs4* (regulator of G protein signalling 4) | Unknown | Repressed | In rat pineal body tissue, validated by ChIP and EMSA | Davies et al. [38] |
| *Atf4* (activating transcription factor 4) | Unknown | Repressed | In rat pineal body tissue, validated by ChIP and EMSA | Davies et al. [38] |
| *Cox6a2* (cytochrome *c* oxidase subunit 6A2) | Unknown | Repressed | In rat pineal body tissue, validated by ChIP and EMSA | Davies et al. [38] |
| *Nr4a1* (nuclear receptor subfamily 4 group A member 1) | Unknown | Repressed | In rat pineal body tissue, validated by ChIP and EMSA | Davies et al. [38] |
| *Dio2* (iodothyronine deiodinase 2) | Unknown | Repressed | In rat pineal body tissue, validated by ChIP and EMSA | Davies et al. [38] |
| *Cd24* (CD24 molecule) | Unknown | Enhanced | In rat pineal body tissue, validated by ChIP and EMSA | Davies et al. [38] |
| *Fra2/Fosl2* (Fos-related antigen 2) | Unknown | Enhanced | In rat pineal body tissue, validated by ChIP and EMSA | Davies et al. [38] |
| *Mt1a* (metallothionein 1A) | Unknown | Enhanced | In rat pineal body tissue, validated by ChIP and EMSA | Davies et al. [38] |
| *Opn1sw* (opsin 1, short wave sensitive) | Unknown | Enhanced | In rat pineal body tissue, validated by ChIP and EMSA | Davies et al. [38] |
| *BSP* (bone sialoprotein) | JunD | Enhanced | In human breast cancer and osteoblast-like cell lines, validated by luciferase reporter assay and EMSA | Detry et al. [39] |
| *TGFB1* (transforming growth factor beta 1) | cJun | Enhanced | In THP-1 monocytic cells, validated by EMSA | Fichtner-Feigl et al. [25] |
| *SOX4* (SRY-box transcription factor 4) | JunD | Enhanced | In adult T-cell lymphoma, validated by ChIP and luciferase reporter assay | Higuchi et al. [40] |
| *Gja1* (gap junction protein α 1/connexion 43) | JunB | Enhanced | SHM cells, validated by luciferase assay | Mitchell et al. [41] |
| *CCCR4* (C-C motif chemokine receptor 4) | JunD/JunB | Enhanced | In adult T-cell lymphoma, validated by luciferase reporter assay and NoShift Transcription Factor Assay | Nakayama et al. [42] |
| *Lama3 (laminin α3A)* | JunD | Enhanced | Mouse keratinocytes, validated by β-galactosidase assay and EMSA | Virolle et al. [43] |
| *LEP* (leptin) | Unknown | Enhanced | Human and mouse adipocytes; validated by quantitative proteomics and ChIP | Wrann et al. [44] |

ChIP: chromatin immunoprecipitation; EMSA: electrophoretic mobility shift assay; SHM: Syrian hamster myometrial cells.

protects it from degradation and prolongs its half-life but if other N-terminal serine residues are phosphorylated, c-jun is directed to ubiquitination [28]. Similarly, MEKK1 has been shown to influences JunB and Fra-2 stability and degradation [30]. Some AP-1 members, such as c-fos and Fra-1 have an intrinsic instable nature, which makes their degradation independent of any ubiquitination [31]. The structural motifs responsible for this intrinsic instability are shared by Fra-2, suggesting that the same mechanism might contribute to Fra-2 protein stability [31]. Hence, the regulation of AP-1 transcription factors has multiple levels including every biological process between protein transcription and protein degradation. The complex interplay of differential gene expression, protein dimerization, activation, diverse transactivating or repressing properties and protein stability of specific AP-1 dimers allows for distinct time-, tissue- and cell-dependent regulation of specific sets of target genes throughout the development and in tissue homeostasis [11]. A summary of target genes directly regulated by Fra-2/AP-1 complexes can be found in Table 1.

### 1.3. In vivo studies on Fra-2 function

In mice, total deletion of Fra-2 causes early postnatal lethality. While no differences are observed between Fra-2 knock-out and wild-type mice at birth, Fra-2 knockout pups suffer from a severe growth defect and die within the first week [45]. The lack of Fra-2 leads to impaired chondrocyte and osteoclast differentiation resulting in compromised matrix cartilage and bone formation [36,46]. In line with this observation, mice overexpressing Fra-2 have increased osteoclast differentiation and are osteosclerotic [36]. Furthermore, ectopic expression of Fra-2 (Fra-2 Tg) results in fibrosis of several organs. Fundamental work by Eferl and colleagues reported fibrogenic activity of Fra-2 predominantly in the lung and the skin, resembling fibrosis seen in patients with systemic sclerosis (SSc), and highlighted the pro-fibrotic role of Fra-2 [5].

The lungs of Fra-2 Tg mice present with strong vascular remodelling accompanied by massive collagen deposition and fibrosis (Fig. 2). Accumulation of αSMA positive cells in the lung parenchyma (myofibroblasts) and in the lung vasculature (smooth muscle cells, SMC) in Fra-2 Tg mice suggests a likely role of Fra-2 in the proliferation of SMC and myofibroblasts. Accordingly, airway smooth muscle cells isolated from Fra-2 Tg mice possess increased basal proliferation without any growth factor stimulation [47]. However, a recent study challenged this idea, as selective overexpression of Fra-2 in SMC and myofibroblasts (α-SMA-rtTA;tetO-Fra-2) did not result in either vascular remodelling or pulmonary fibrosis but alveolar enlargement with an emphysema like phenotype [48]. While fibrosis might be a continuous attempt to repair the emphysema like phenotype in Fra-2 Tg mice, it cannot be excluded that overexpression of Fra-2 in other cells, such as epithelial cells could contribute to pulmonary fibrosis independently from the phenotype induced by the Fra-2 overexpressing myofibroblasts. However, Fra-2 inactivation in alveolar type 2 cells does not have protective effects in a bleomycin-mouse model, indicating that Fra-2 expression in these cells is not required for the development of lung fibrosis [49]. Another important phenotype in Fra-2 Tg mice is inflammatory cell infiltration, which presents as a strong perivascular and peribronchial accumulation of inflammatory cells and precedes the development of fibrosis [17,47]. Indeed, several studies have pointed out that while some cell populations such as B- and T-cells do not play a role in Fra-2 induced fibrosis others such as macrophages promote fibrosis development in mice [5,49]. The phenotypes observed in diverse Fra-2 transgenic mice are briefly described in Table 2.

## 2. Fra-2 in chronic lung diseases

Fra-2 has a crucial role in the regulation of cell growth and differentiation, as well as in tissue homeostasis where it integrates intra- and extracellular cues. Therefore, if aberrantly expressed it may be involved in the development of chronic diseases, especially in organs where it is normally expressed, such as the lung [14]. In healthy adult lungs immunostaining identified strong Fra-2 expression in some bronchial epithelial cells, vascular smooth muscle cells and in alveolar macrophages [5,17,34,49], however, it cannot be excluded that all cell types express some level of Fra-2, as it is an integral part of normal homeostatic signalling. In fibrotic lung tissue Fra-2 can be detected in bronchial and alveolar epithelial, endothelial and vascular smooth muscle cells, inflammatory cells as well as mesenchymal cells [5,34,49]. Several studies have shown enhanced Fra-2 expression and activation in diverse lung diseases; elevated levels of Fra-2 were reported in fibrotic lungs (and skin) of patients with systemic sclerosis (SSc) [5,18,53], interstitial lung disease (ILD) and idiopathic pulmonary fibrosis [5,49], in the vasculature of pulmonary hypertension patients [17], and in pulmonary macrophages from chronic obstructive pulmonary disease (COPD) patients [54] (Table 3). However, how Fra-2 mechanistically contributes to the development of these chronic lung diseases is not yet fully elucidated and will be discussed in the following sections.

### 2.1. Remodelling of lung parenchyma

Fibrosis is the formation of a scar tissue due to an abnormal reparative process and excessive extracellular matrix (ECM) production [55,56]. The increased expression of Fra-2 in lungs of pulmonary fibrosis patients (IPF as well as SSc-associated pulmonary fibrosis) highlights the importance of Fra-2 as a key pro-fibrotic factor in chronic lung disease [5,57]. In the bleomycin-induced mouse model of lung fibrosis, Fra-2 is upregulated in mesenchymal cells and macrophages. Local inactivation of Fra-2 by adenoviral delivery of Cre recombinase and Cre-induced knockout ameliorated the development of fibrosis [49]. Fra-2 Tg mice demonstrated a fibrotic phenotype with accumulation of ECM producing myofibroblasts and honeycombing similar to human idiopathic pulmonary fibrosis (IPF). These fibrotic changes in Fra-2 mouse lungs led to a decrease in inspiratory capacity and pulmonary compliance [34]. Fra-2 Tg lungs showed also perivascular and peribronchial inflammatory infiltrates which are more pronounced features of immune mediated fibrosis such as non-specific interstitial pneumonia (NSIP) [5]. The aspects of inflammation in Fra-2 dependent fibrosis development is discussed in the dedicated chapter.

Fra-2 has been shown to induce Col1a2 in osteoblasts [36] and transcriptome analysis of Fra-2 Tg mice showed that ECM and cell-adhesion molecules are the most differentially regulated genes compared to WT littermates [48]. Another study reported downregulation of basal Col1a1, Col1a2 and Col5a1 expression upon Fra-2 knockdown in dermal fibroblasts, indicating a role of Fra-2/AP-1 in the regulation of collagen production [18]. We have previously shown that Fra-2 Tg mice have higher levels of the matrix metalloproteinase meprin β [17], which is expressed by epithelial and inflammatory cells [58]. Meprin β cleaves the N- and C-terminal domains of pro-collagen, thereby, releasing mature collagen [59]. Hence, epithelial cells by producing meprin β could contribute to the maturation of collagen expressed by fibroblasts. Additionally, Fra-2 has been shown to mediate expression of the collagen-crosslinking enzyme lysyl oxidase-like 4 (Loxl4), which is secreted into the extracellular space where it contributes to collagen deposition, assembly and thus maturation [60]. However, differences exist between in vitro and in vivo studies: Although increased collagen deposition was observed in vivo, pulmonary fibroblasts isolated from Fra-2 Tg mice did not show higher collagen production at baseline or upon stimulation compared to fibroblasts isolated from WT littermates controls [5]. This indicates that Fra-2 does not lead to increased ECM deposition in a cell-autonomous manner, but that pulmonary fibrosis development in these mice is more complex than aberrant ECM production by mesenchymal cells due to Fra-2 overexpression. A recent study showing fibroblast to myofibroblast differentiation through Fra-2-dependent Col6 expression in myeloid cells highlights this complex interplay between different cell-types and the contribution of Fra-2 in fibrotic processes [49].

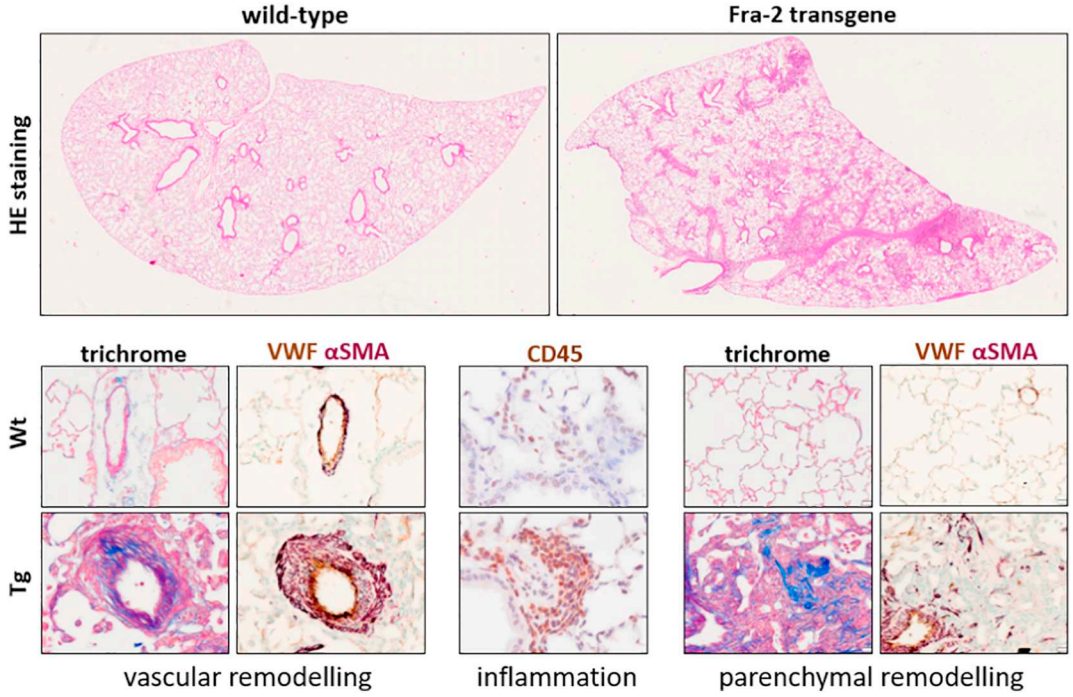


**Fig. 2.** Pulmonary remodelling in Fra-2 overexpressing mice.
In mice, Fra-2 overexpression induces pronounced inflammatory cell recruitment and structural alterations, hematoxylin and eosin (HE) staining (upper panel). Transgenic (Tg) overexpression results in vascular remodelling with collagen deposition in the vessel wall (shown by Masson's trichrome staining with collagen in blue) and increased vessel muscularisation (highlighted by dual immunohistochemistry with the endothelial cell marker von Willebrand factor (VWF) and the smooth muscle cell marker α-smooth muscle actin (αSMA)). Vascular remodelling is accompanied by perivascular CD45-positive inflammatory infiltrates. At later stages, remodelling processes in the parenchyma occur with elevated collagen deposition and occurrence of α-SMA-positive myofibroblasts in the parenchyma of Fra-2 Tg mice.

Fra-2 also seems to be an important player in ischemia reperfusion-induced fibrosis, as oxygen-induced TGFβ expression in cardiac fibroblasts is Fra-2-dependent [61]. TGFβ is a key regulator of scar formation and fibrosis [62] and activation of TGFβ signalling leads to the production of ECM molecules, especially collagens [63]. Fra-2 is tightly linked with TGFβ signalling as it can on the one hand induce TGFβ expression [25] and on the other hand act downstream of TGFβ signalling [60,64]. For example, TGFβ induced laminin expression is mediated by Fra-2 [43]. All these studies strongly indicate a link of Fra-2 to the development of fibrosis. Nevertheless, taken into consideration the complex regulation of Fra-2, which is dependent on dimerization partner, cell type, stimuli, the cellular milieu and even on cell cycle phases, one has to be careful in generalizing Fra-2 fibrogenic effects described in only one organ or cell type.

### 2.2. Vascular remodelling

Vascular remodelling is a hallmark of pulmonary hypertension (PH) and includes proliferation of vascular cells and deposition of ECM in the vessel wall [65]. Prior to the development of pulmonary fibrosis, Fra-2

**Table 2**
Different pulmonary phenotypes identified on global or cell-specific Fra-2 overexpressing or knockout mice.

| Model | Described phenotype | Reference |
|---|---|---|
| Fra 2 Tg mice (global overexpression) | • Increased pulmonary pressure<br>• Increased vascular collagen | Biasin et al. [17] |
| | • Lung fibrosis<br>• Pronounced vascular remodelling<br>• Enhanced inflammation | Eferl et al. [5] |
| | • Perivascular inflammation<br>• Vascular Remodelling<br>• Lung fibrosis | Maurer et al. [50] |
| | • Airway hyperreactivity- non allergic asthma | Gungl et al. [47] |
| | • Restrictive lung function<br>• Lung fibrosis<br>• Perivascular inflammation | Birnhuber et al. [34] |
| $Fra\text{-}2^{Ob\text{-}tet}$ (Fra2 overexpression in osteoblasts) | • Systemic inflammation<br>• Increased sensitivity to lung injury | Luo et al. [51] |
| $\alpha\text{-SMA-Cre}^{ER}$;$Tgfbr2^{flox/flox}$-Fra2 (global overexpression with Tgfbr2 knock out in SMA+ cells) | • Improved remodelling<br>• Decreased SMC proliferation | Tsujino et al. [52] |
| α-SMA-rtTA;tetO-Fra-2 (Fra2 overexpression in SMA+ cells) | • Emphysema-like phenotype | Tsujino et al. [48] |
| Lyz2-Cre;$Fra2^{flox/flox}$ (myeloid-specific knockout) | • No phenotype without additional challenge<br>• Ameliorated bleomycin-induced lung fibrosis | Ucero et al. [49] |
| SPC-Cre;$Fra2^{flox/flox}$ (ATII-specific knockout) | • No phenotype without additional challenge<br>• No effect on bleymcin-induced lung fibrosis | Ucero et al. [49] |

**Table 3**
Fra2 expression in chronic lung diseases.

| Disease | Regulation of Fra-2 | Reference |
|---|---|---|
| Systemic sclerosis (SSc - skin) | Up-regulation and increased nuclear translocation | Reich et al. [18], Maurer et al. [53] |
| Systemic sclerosis (SSc - lung) | Up-regulation | Eferl et al. [5], Birnhuber et al. [34] |
| Idiopathic pulmonary fibrosis (IPF) | Up-regulation | Eferl et al. [5], Ucero et al. [49] |
| Pulmonary hypertension (PH) | Up-regulation in vessels | Biasin et al. [17] |
| Chronic obstructive pulmonary disease (COPD) | Up-regulation in monocyte derived macrophages upon cigarette smoke stimulation | Kent et al. [54] |

Tg mice develop vascular changes with increased neomuscularisation of small parenchymal vessels (50 μm–100 μm diameter) and also severe remodelling of larger vessels (up to 250 μm diameter), including intimal thickening and vascular obliteration as observed in patients with PH [5,17]. We have previously shown that silencing of Jun/Fra-2 family dimers in human pulmonary artery smooth muscle cells (hPASMC) in vitro leads to decreased PDGF-BB induced proliferation [17]. Similar results were obtained by other studies where AP-1 was responsible for proliferation of vascular smooth muscle cells [66,67]. These findings imply a direct effect of Fra-2 on proliferation of vascular SMC, however specific overexpression of Fra-2 in α-SMA-positive cells does not lead to increased muscularisation of vascular remodelling in vivo [48]. Furthermore, over-expression of Fra-2 can cause cell-type dependent effects. Endothelial cells in the skin of Fra-2 Tg mice have increased apoptosis [53], while in lung endothelial cells, apoptosis was only observed in the late fibrotic stages of the disease [5]. Cumulatively these findings indicate that endothelial cell apoptosis is probably not the cause of vascular remodelling in Fra-2 Tg mice. However, further studies are warrant to delineate the exact role of Fra-2 in lung endothelial cells.

Fra-2 overexpression was associated with vascular collagen deposition and enhanced meprin β expression. Meprin β was transcriptionally regulated by TGFβ1 and Fra-2/AP-1 in PASMC [17]. As a potent pro-fibrotic factor TGFβ also plays a crucial role in the development of PH. Recent studies have proven the importance of TGFβ in PH by blockade of its pathway in Fra-2 Tg mice. Global TGFβ blockade in Fra-2 Tg mice ameliorated vascular remodelling and smooth muscle thickening, without affecting pulmonary fibrosis and even with adverse effects regarding inflammation [52]. Smooth muscle cell specific deletion of the TGFβ receptor 2 (TGFβR2) decreased smooth muscle cell expansion, indicating that TGFβ signalling directly contributes to vascular remodelling in Fra-2 Tg mice [52]. Together these finding suggest that Fra-2 contributes to medial thickening by affecting smooth muscle cell proliferation and by influencing endothelial cell apoptosis and ECM matrix deposition. It is currently unknown which Jun components dimerize with Fra-2 and mediate these effects. We have reported a possible interaction in vitro with JunB [17], however in vivo these phenotypes are probably not mediated by one single dimerization partner but rather by dynamic interaction with diverse components and possible formation of several complexes.

A frequent consequence of pulmonary hypertension is right ventricular hypertrophy and impaired cardiac function [68]. In SSc patients high levels of Fra-2 expression are not only found in the lungs, but also in myocardial tissue. Similarly, Fra-2 Tg mice exhibit decreased capillary density, elevated inflammatory infiltration, myofibroblast accumulation and collagen deposition in the myocardium, mimicking all aspects of SSc-related cardiac myopathy [69,70]. Already during embryogenesis Fra-2 plays a role in cardiac development as aberrant regulation of Fra-2 in zebrafish disturbs ventricular morphogenesis and growth due to impaired cardiomyocyte differentiation [71]. Thus Fra-2 and its downstream mediators could be crucial contributors to cardiomyopathy associated with chronic lung diseases.

### 2.3. Asthma and airway remodelling

Asthma is a chronic lung disease, which is characterized by airway remodelling and hyperresponsiveness, and chronic inflammation. Little is known about AP-1 or Fra-2 in the development of asthma, although bronchial fibroblasts from asthmatic patients showed increased AP-1 DNA binding compared to fibroblasts from non-asthmatic controls [72]. Treatment of ovalbumin (OVA)-induced asthmatic mice with AP-1 decoy oligonucleotides led to downregulation of Th2 cytokines and ameliorated the pathophysiological features induced by OVA [73]. However, studies on specific AP-1 subunits in asthma are scarce and mostly limited to c-fos.

We recently demonstrated that Fra-2 overexpression not only leads to remodelling in the vascular and parenchymal compartments of the lung, but that it induces changes of airway/bronchial architecture [47]. Overexpression of Fra-2 in mice leads to sub-epithelial fibrosis and increased smooth muscle thickness in the airways, accompanied by peribronchial inflammatory infiltrates and airway hyperresponsiveness [47]. Importantly, this phenotype is induced without the need of exogenous antigens and therefore, represents one of the few models to investigate intrinsic asthma. The aforementioned study highlights how Fra-2 could be a focal factor for several features of asthmatic airway disease: transcriptomic and in silico transcription factor analysis indicated that Fra-2 is involved in the transcriptional regulation of several genes involved in asthma-pathways like mucus production, ECM deposition and inflammation, as well as in asthma susceptibility [47].

Fra-2 can influence mucus production on multiple levels: It induces the expression of transcription factors leading to differentiation of mucus-producing goblet cells (e.g. Spdef, Foxa3), the mucin gene Muc5AC, and also factors crucial for the correct hydration and secretion of mucus (e.g. Clca1) [47]. Similarly in humans, Fra-2 has been shown to be important for the production of airway mucin, as smoke-exposed epithelial cells increase their MUC5AC expression via the JunD/Fra-2 dimer [74]. Furthermore, transcriptomic analysis showed increased expression of ECM genes such as Col1a2, Col6a5 and Fibronectin, and ECM-regulating genes (MMP12, TIMP1) highlighting its role in regulating extracellular matrix deposition [18,47]. We previously reported a direct pro-proliferative role of Fra-2 in airway smooth muscle cells [47], however, whether SMC proliferation is the driving force or only a contributing factor causing the remodelling is doubtful. More likely, the cumulative effects of Fra-2 on collagen deposition, cell proliferation, inflammation and mucus production give rise to the observed phenotype.

## 3. Fra-2 as a regulator of inflammation

In all the above-mentioned disorders inflammatory processes have a crucial role [56,75–77]. Several studies have shown the interdependence between inflammation and Fra-2 [5,47,51]. Strong nuclear expression of Fra-2 was observed in patients with immune-mediated pulmonary fibrosis and interstitial pneumonia [5,57], while overexpression of Fra-2 in mice results in systemic inflammation [51], and inflammatory infiltrates in the skin [53,57] and the lung [5,47,52].

Fra-2 overexpressing mice display a hyper-inflammatory state with strong peri-vascular, peri-bronchial and alveolar recruitment of numerous inflammatory cell populations (Fig. 3A). This inflammation is characterized mostly by increased numbers of CD4 and CD8 positive lymphocytes and eosinophils [47]. The influx of inflammatory cells in the bronchoalveolar lavage of Fra-2 Tg mice strongly correlates with declined lung function [34]. In conjunction with this inflammatory cell influx, higher levels of multiple cytokines are also observed. These

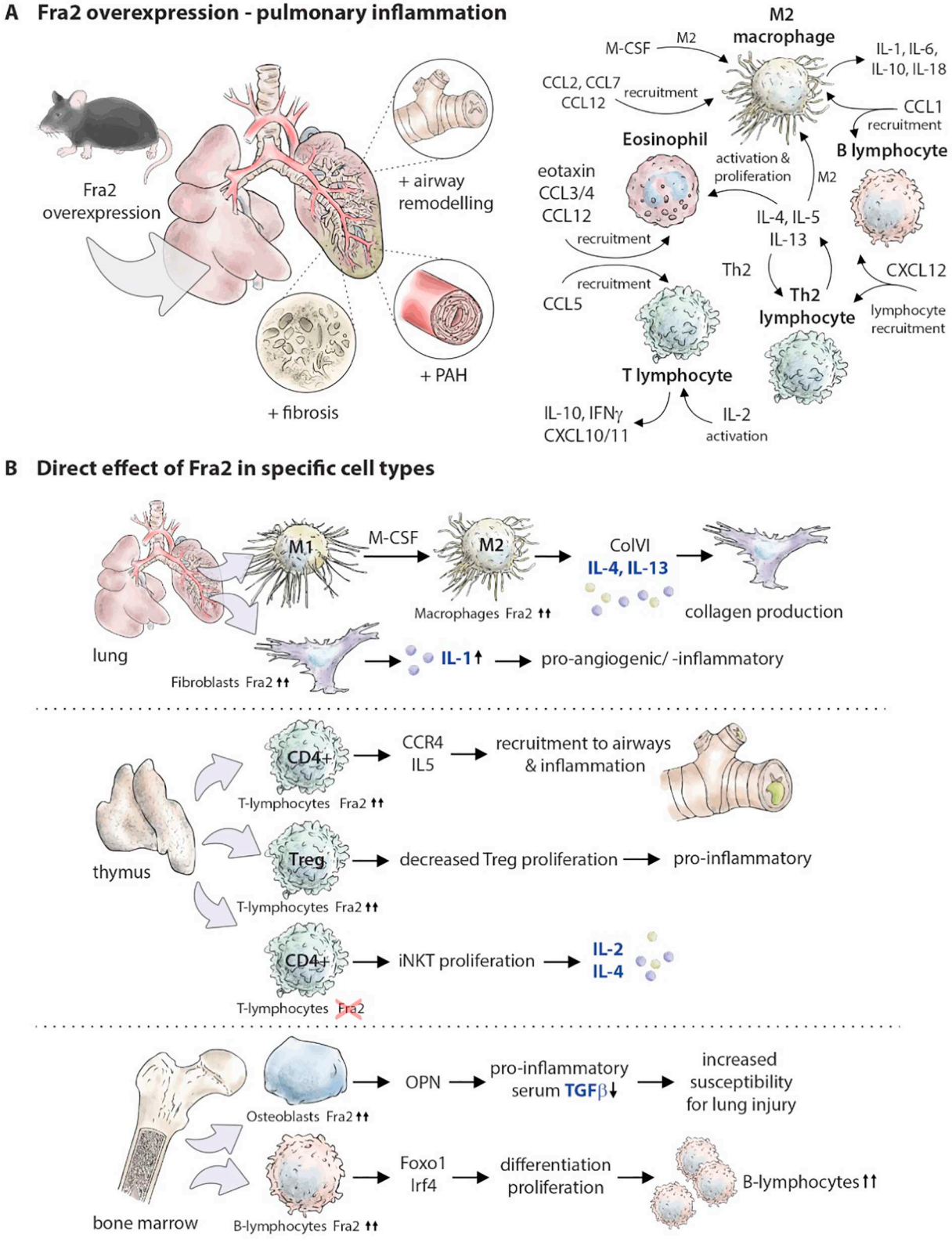


**Fig. 3.** Fra-2 as a regulator of inflammation.

A) Schematic representation of pulmonary remodelling and inflammatory pathways activated in Fra-2 overexpressing (Tg) mice. Several chemokines of the C-C motif chemokine ligand (CCL) an the C-X-C- motif chemokine ligand (CXCL) families lead to recruitment of inflammatory cells to the lungs of Fra-2 Tg mice. These inflammatory infiltrates are characterized by high levels of eosinophils, T- and B-lymphocytes and alternatively activated, pro-fibrotic macrophages (M2). Inflammatory mediators such as IL-2, M-CSF and the Th2 cytokines interleukin (IL)-4, -5, and -13 lead to activation of T-lymphocytes, Th2 and M2 polarization and priming/activation of eosinophils. B) Pro-inflammatory Fra-2 effects in specific cell types. Fra-2 activity is essential for the expression of pro-fibrotic factors in alternatively activated macrophages and induces IL-1 production in parenchymal fibroblasts. In T-cells, Fra-2 upregulates CCR4 and IL-5 expression, leading to airway inflammation, as well as decreasing regulatory T-cell (Treg) proliferation. Deletion of Fra-2 elevates numbers of invariant natural killer T-cells (iNKT). In the bone marrow, Fra-2 activates B-lymphocyte differentiation and proliferation, and induces osteopontin (OPN) expression in osteoblasts, thereby causing a systemic inflammatory state with increased susceptibility to lung injury. Inflammatory mediators which in turn regulate Fra-2 expression and activity are highlighted blue.

include IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, IL-18, macrophage colony-stimulating factor (M-CSF); and the chemokines, CCL2/MCP-1, CCL7/MCP-3, CCL12/MCP-5, CCL5/MIP-1 and eotaxin/CCL11 [5,47,70]. M-CSF is an important growth factor for monocytes and macrophages and promotes their polarization into alternatively activated (M2) macrophages. M2 macrophages are commonly regarded as pro-fibrotic and implicated in pulmonary [78,79], skin [80], liver [81] and kidney [82] fibrosis. They are also significantly increased in the skin [70] and in the lungs of Fra-2 Tg mice [49] and their numbers increase when fibrosis is deteriorating [34]. Interestingly it has been shown that Fra-2 is not essential for the polarization of M2 macrophages, but that its expression in these macrophages is essential for the production of pro-fibrotic factors stimulating fibroblast to myofibroblast differentiation [49] (Fig. 3B).

In the late fibrotic stage of Fra-2 Tg mice, high levels of IL-1, IFN-γ, CCL1, CCL3, CCL4, CXCL2, CXCL10 and CXCL11 are observed [5]. A number of these chemokines are implicated in fibrosis, cell migration and collagen production. IL-1 and CXCL2 act pro-fibrotic by enhancing the inflammatory response and angiogenesis [83,84], whereas others, such as CXCL10, CXCL11 and IFN-γ can supress collagen production [84] and are important for resolution of fibrosis [85]. Higher expression of these anti-fibrotic factors may seem at first counter-intuitive, however, they may be expressed as a measure to counteract the ongoing fibrosis development.

TGFβ is an important pro-fibrotic cytokine in multiple organs. Global blockade of TGFβ in Fra-2 TG mice decreases pathologic remodelling but increases inflammation [52]. TGFβ can act as a potent anti-inflammatory factor and is important in the induction and regulation of regulatory T cells [86] and the control of autoimmunity [87]. There are indications that Fra-2 overexpression interferes with the differentiation and function of regulator T cells (Tregs) [88] (Fig. 3B). Defective or decreased Tregs are associated with several chronic lung diseases, such as asthma [89] and COPD [90] and are important for tissue repair and restriction of inflammation in acute lung injury [91]. In paediatric asthma, Tregs are decreased and not able to suppress Th2 mediated inflammation, however, their function can be restored by treatment with inhaled corticosteroids [89].

In Fra-2 Tg mice the presence of eosinophils, T cells and associated Th2-cytokines (e.g. IL-5 and IL-13), indicate a strong Th2 phenotype. In human T-cells, de novo synthesis of Fra-2 was shown to be required for IL-5 synthesis [92]. Furthermore, Fra-2 expression in T cells have been shown to induce expression of CCR4 chemokine receptor [79], which activation is important for T cell recruitment in airways of asthmatic patients [80]. Although currently untested, this mechanism likely contributes to the Fra-2 Tg phenotype. Th2 mediated inflammation underlies atopic diseases such as asthma or dermatitis [93,94]. In Fra-2 Tg mice treatment with IL-13 neutralizing antibodies or inhaled corticosteroids, attenuates several features of pathological remodelling, including airway remodelling and hyper-reactivity, indicating a causal role for Th2 inflammation in the Fra-2 disease phenotype [47]. Additionally, IL-13 may further contribute to pathological remodelling via binding the IL13Ra2 receptor and the induction of TGFβ-production [25]. However, following IL-13 blockage the inflammatory phenotype shifts to a neutrophilic inflammatory phenotype with high levels of IL-17 [47]. Therefore, suitable treatment strategies for distinct asthma endotypes have to be chosen carefully, since such adverse effects might be detrimental for patients with mixed eosinophilic/neutrophilic asthma [47].

Not only over-expression of Fra-2 but also its deletion alters and affects the inflammatory status in mice. Selective deletion of Fra-2 in CD4 expressing cells (CD4cre/Fra-$2^{f/f}$ mice) results in elevated numbers of invariant natural killer T (iNKT) cells in the thymus and periphery without affecting the numbers of CD4, CD8 cells or Tregs. These iNKT cells show enhanced proliferation and secretion of IL-2 and IL-4 [95] leading to mucus and type 2 cytokine production [96,97].

Fra-2 regulates and increases differentiation and proliferation of B-cells, by inducing expression of the transcription factors Foxo1 and Irf4 [98] (Fig. 3B). Consistently, Fra-2 overexpressing mice have increased numbers of B cells [47]. Abnormal aggregates of B cells have been shown in IPF patients, where they produce antibodies and pro-fibrotic/pro-inflammatory cytokines, such as TGFβ and IL-6, and therefore contribute to the pathogenesis of pulmonary fibrosis [99]. Nevertheless, the pathogenic phenotype caused by Fra2 overexpression is not solely due to the influence on bone marrow derived inflammatory cells. Pulmonary fibrosis still develops in Fra-2 Tg mice reconstituted with wild-type bone marrow or in Fra-2 Tg mice lacking functional T and B lymphocytes (Fra-2 Tg/$Rag2^{-/-}$). These results indicate that B and T lymphocytes are dispensable in the pathogenesis of pulmonary fibrosis in Fra-2 Tg mice [5].

Fra-2 has a multi-factorial role in the bone marrow where it is a key regulator of bone development, bone mineral maturation and osteoblast differentiation [36]. Specific overexpression of Fra-2 in osteoblasts (Fra-$2^{Ob\text{-}tet}$) induces osteopontin (OPN) transcription and thereby polarizes MSCs (mesenchymal stem cells) towards an inflammatory phenotype [51]. This phenotype is associated with systemic inflammation with decreased serum levels of the anti-inflammatory cytokine IL-10 and increased mobilisation of monocytes and myeloid progenitors into the blood. Additionally, Fra-$2^{Ob\text{-}tet}$ mice showed more severe lung injury and inflammatory infiltration following LPS treatment [51].

Finally, as mentioned previously the expression of Fra-2 can be activated by several inflammatory cytokines such as IL-2, IL-4 and IL-13, which act via JAK3/STAT5 signalling [25,49,100]. IL-1, IL-17 and TNFα can enhance the expression of Fra-2 and its nuclear localization [34,101], suggesting a positive feedback loop in which Fra-2 acts pro-inflammatory by activating transcription of inflammatory mediators, which in turn stimulate expression of Fra-2. Taken together, Fra-2 appears to be at the centre of a positive feedback loop, where Fra-2 can be induced by several inflammatory stimuli and in turn activates the transcription of inflammatory mediators, which feedback in the activation of Fra-2.

## 4. Therapeutic implications

Despite Fra-2 having a central role in the development of chronic lung disease it is difficult to envision Fra-2 as potential target due to its multiple interactions. However, a recent study showed an improved phenotype in several models of pulmonary fibrosis upon treatment with an AP-1 inhibitor and claimed that AP-1 inhibition should be considered as a therapeutic target for the treatment of pulmonary fibrosis [49]. Additionally, understanding the mode of action of Fra-2 can aid the development of novel therapeutic strategies.

Several treatment strategies such as growth factor inhibition, regulation of ECM turnover and anti-inflammatory treatments have been tested and evaluated in Fra-2 Tg mice (Table 4). While general anti-inflammatory treatment using inhaled corticosteroids proved to be beneficial [47], inhibition of specific inflammatory pathways has to be handled with care. When applying IL-13 neutralizing antibodies, the asthmatic phenotype of Fra-2 Tg mice improved by decreasing bronchial remodelling and dampening pulmonary inflammation, similar to treatment with corticosteroids. However, although the overall phenotype of the mice was ameliorated, an inflammatory shift towards increased Th17 response and neutrophils was observed [47]. Blockade of IL-1 signalling using the receptor antagonist anakinra worsened pulmonary fibrosis and increased the numbers of inflammatory cells in the lungs of Fra-2 Tg mice [34]. This deterioration was accompanied by increased expression of M2 markers in the lung and accumulation of M2 macrophages [34].

The fibrotic phenotype of the mice was ameliorated by Abatacept, a fusion protein blocking T-cell interaction with stimulatory co-receptors and thereby T-cell activation [104]. In addition to blocking T-cell proliferation, Abatacept led to a decrease in alternatively activated M2 macrophage numbers and decreased lung density and vascular remodelling [104]. Interestingly, also treatment with the tyrosine kinase

**Table 4**
Treatments applied on Fra2 Tg mice and consequent effects.

| Treatment | Lung phenotype | Reference |
|---|---|---|
| T-5224 (AP-1 inhibition) | • Improved lung function<br>• Decreased lung fibrosis | Ucero et al. [49] |
| pan-PPAR agonist IVA337 | • Reduction of fibrosis<br>• Reduction of PH | Avouac et al. [102] |
| MMP10 neutralizing antibody | • Reduction of PH | Avouac et al. [103] |
| Nilotinib (Tyrosine kinase inhibitor) | • Decreased lung fibrosis<br>• Reduced remodelling | Maurer et al. [50] |
| Nintedanib (Tyrosine kinase inhibitor) | • Decreased lung fibrosis<br>• Reduced remodelling | Huang et al. [70] |
| Global TGFβ inhibition (1D11) | • Increased inflammation<br>• No change in fibrosis<br>• Reduced remodelling | Tsujino et al. [52] |
| Abatacept (T cell activation blockage) | • Decreased lung fibrosis<br>• Reduced vascular remodelling<br>• Reduction of PH | Boleto et al. [104] |
| Budesonide (corticosteroids) | • Reduced airway hyperresponsiveness<br>• Less inflammation<br>• Less airway remodelling | Gungl et al. [47] |
| IL-13 neutralizing antibody | • Reduced airway hyperresponsiveness<br>• Less inflammation<br>• Less airway remodelling | Gungl et al. [47] |
| Anakinra (IL-1R antagonist) | • Increased Th2 inflammation<br>• Worsened lung fibrosis<br>• No changes in vascular remodelling | Birnhuber et al. [34] |

inhibitors Nilotinib and Nintedanib or a pan-PPAR agonist led to decreased levels of macrophages and diminished pulmonary remodelling in the parenchyma as well as in the vasculature [50,70,102]. Although Fra-2 was shown to be essential for the expression of pro-fibrotic factors in M2 macrophages, it did not influence macrophage polarization itself [49], indicating that decreased levels of M2 macrophages might be a key in successfully treating the fibrotic phenotype of Fra-2 Tg mouse lungs.

Interestingly, different pathways seem to be involved in the Fra-2 induced remodelling processes of the lung vasculature and the parenchyma. For example, blockade of IL-1 signalling worsened pulmonary inflammation and fibrosis, but did not affect vascular remodelling or pulmonary pressure [34]. In contrast, neutralizing matrix metalloproteinase-10 (MMP-10) did not influence the severity of pulmonary fibrosis, however had a beneficial effect of the development of vascular remodelling and even reversed established PH in Fra-2 Tg mice [103]. Furthermore, the inhibition of TGFβ signalling improved vascular remodelling while leaving pulmonary fibrosis unaffected and even deteriorating pulmonary inflammation [52]. Specific deletion of TGFβ signalling however improved vascular remodelling without negatively affecting fibrosis or inflammation [52]. Cumulatively, these studies show that successful treatment of pulmonary remodelling processes requires a better understanding of the complex underlying pathomechanisms and more selective targeting to avoid detrimental side effects.

### 4.1. Limitations of the Fra-2 Tg mouse model

Global ectopic overexpression of Fra-2 leads to a complex phenotype affecting multiple cell-types and tissues. Therefore, the major limitation of this model is a lack of cell-type specific information, and whether observed effects are directly or indirectly controlled by Fra-2. Another limitation is the high phenotype variability of Fra-2 Tg mice, which might be due to modulation of the inserted transgene by the genetic environment. The Fra-2 induced phenotype resembles a co-morbidity model combining several diseases such as SSc-associated pulmonary fibrosis, hypertension and asthma [5,34,47]. While this complexity gives valuable information and is comparable to human disease, it can complicate the analysis of specific pathomechanisms (Fig. 4).

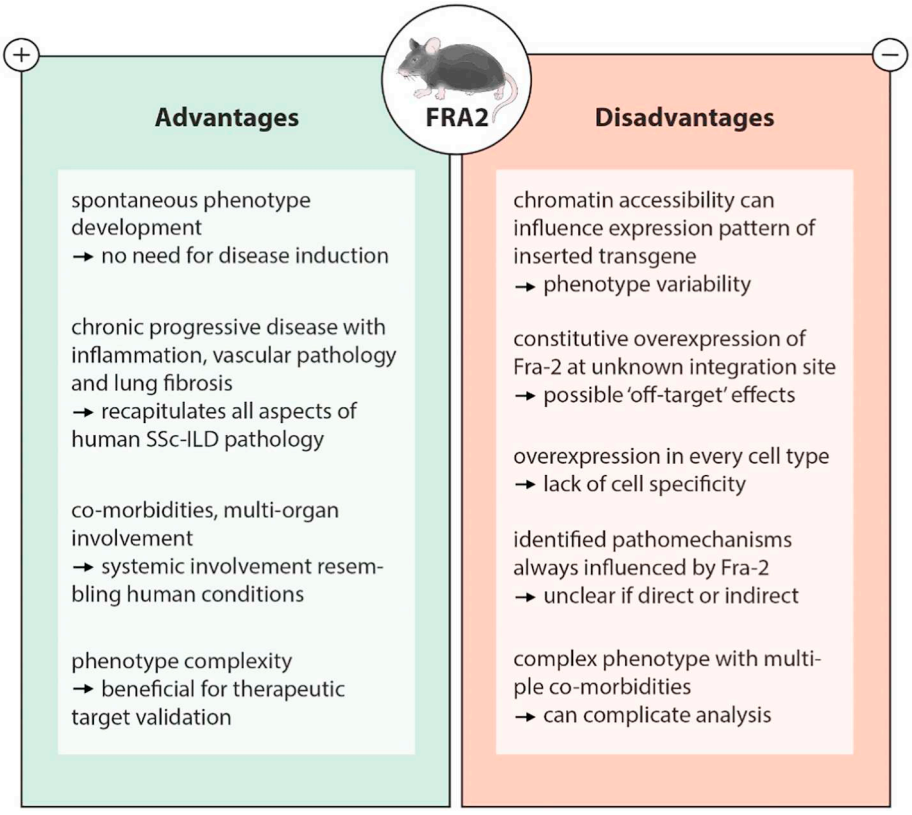


**Fig. 4.** Advantages and limitations of the Fra-2 overexpressing (Tg) mouse model.

Nevertheless, this mouse model recapitulates many aspects of human pulmonary pathology, including inflammation, vascular remodelling and fibrosis. Thus, the Fra-2 Tg mouse model can be a valuable tool in deciphering key pathomechanisms of pulmonary remodelling and in evaluating potential future therapeutics. However, more research is needed to clearly delineate and explain the source of aberrant Fra-2 expression and to understand how Fra-2 contributes to disease pathogenesis in chronic lung diseases.

## Declaration of Competing Interest

None.

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