Thy-1/β3 Integrin Interaction-Induced Apoptosis of Dermal Fibroblasts Is Mediated by Up-Regulation of FasL Expression

Journal of Investigative Dermatology (2016) **136,** 526–529; doi:10.1016/j.jid.2015.10.063

TO THE EDITOR

Control of the balance between cell proliferation, differentiation, and apoptosis of fibroblasts is crucial for maintaining tissue homeostasis, physiological wound healing/scar formation, and prevention of tissue fibrosis or tumor progression (Eckes et al., 2014; Gauglitz et al., 2011; Igney and Krammer, 2002; Varga and Abraham, 2007).

Thy-1 is a glycosylphosphatidyl-anchored cell surface protein expressed on activated endothelial cells, neuronal cells, and fibroblasts. Recently, we reported a contribution of Thy-1 to the maintenance of skin homeostasis by suppressing proliferation and promoting apoptosis of dermal fibroblasts via interaction with β3 integrins (Schmidt et al., 2015). Thus, primary dermal fibroblasts from Thy-1–deficient (Thy-$1^{-/-}$) mice (Nosten-Bertrand et al., 1996) displayed increased proliferative capacity and in parallel significantly fewer apoptotic cells compared to wild-type (wt) fibroblasts, resulting in enhanced cell growth. Thy-1–mediated effects were strongly dependent on the interaction with β3 integrins. In addition, suppression of cell growth of Thy-$1^{-/-}$ fibroblasts by immobilized recombinant Thy-1 suggests that Thy-1 transmits the signal in trans into β3 integrin–expressing cells (Schmidt et al., 2015). Currently, whether Thy-1 might act with β3 integrins on the same cell remains unknown. Here we aimed to elicit the mechanisms of Thy-1/β3 integrin–mediated control of programmed cell death in fibroblasts.

Apoptosis is one important mechanism in maintaining tissue homeostasis and is triggered mainly by the extrinsic pathway via transmembrane receptor–mediated interactions. The well-characterized Fas ligand (FasL, CD95L) belongs to the tumor necrosis factor family and induces apoptosis by Fas receptor (Fas, CD95) trimerization and subsequent caspase activation (Waring and Mullbacher, 1999).

Therefore, we analyzed FasL and Fas expression in mouse fibroblasts dependent on Thy-1/β3 integrin interaction. Fibroblasts from wt and Thy-$1^{-/-}$ mice expressed β3 integrin in vitro and in vivo (see Supplementary Figure 1 online). Interestingly, wt fibroblasts showing enhanced apoptosis expressed higher levels of FasL on mRNA (Figure 1a) and protein levels (Figure 1b and f) compared to Thy-$1^{-/-}$ fibroblasts. In contrast, Fas expression was not different in wt and Thy-$1^{-/-}$ fibroblasts (Figure 1c and d). In accordance, an association of Thy-1 and FasL expression has been observed on lung fibroblasts from bleomycin-treated wt and Thy-$1^{-/-}$ mice (Cohen et al., 2009). Here we show that Thy-1 expression on dermal fibroblasts is sufficient to control basal FasL expression independent of exogenous stimulation. Next, blocking of β3 integrins but not β1 integrins on wt fibroblasts resulted in down-regulation of FasL mRNA and protein expression to the level of FasL in Thy-$1^{-/-}$ fibroblasts (Figure 1e and f). In contrast, blocking β3 integrins did not affect FasL expression in Thy-$1^{-/-}$ fibroblasts. These results indicate that the interaction of Thy-1 with β3 integrins regulates FasL expression on dermal fibroblasts.

To show whether Thy-1/β3 integrin–mediated control of FasL expression is responsible for Thy-1–mediated regulation of apoptosis, wt and Thy-$1^{-/-}$ fibroblasts were incubated with function-blocking antibody against FasL. Next, proliferation rate and apoptosis were determined (Figure 1g and h). Blocking of Fas/FasL–induced apoptosis in wt fibroblasts completely reversed Thy-1–mediated effects on cell proliferation and apoptosis, whereas no effects were observed in Thy-$1^{-/-}$ fibroblasts. Consequently, cell growth measured by metabolic conversion of tetrazolium salt XTT was increased in wt fibroblasts after blocking of FasL (Figure 1i). Our data indicate that the interaction of Thy-1 with β3 integrin stimulates FasL expression, resulting in enhanced apoptosis and reduced cell growth of dermal fibroblasts. However, differences in proliferation rate between wt and Thy-$1^{-/-}$ fibroblasts were detected 24 hours after seeding the cells, whereas Thy-1–mediated apoptosis could be observed from day 3 in culture. Hence, we suppose a preceding, additional FasL-independent Thy-1–mediated effect on fibroblast proliferation.

Cohen et al. (2009) described a direct regulation of FasL expression by Thy-1 activation. To get insights on whether induction of FasL on dermal fibroblasts is a direct result of Thy-1 activation, wt fibroblasts were cultured on increasing concentrations of immobilized anti–Thy-1 antibody (clone G7), which has been previously demonstrated to induce FasL expression in T cells (Kojima et al., 2000). In contrast to Cohen et al. (2009), direct activation of Thy-1 using crosslinking anti–Thy-1 antibody (G7) did not affect apoptosis and consequently cell growth in wt fibroblasts (see Supplementary Figure 2 online). Taken together, reduced cell growth based on enhanced apoptosis and decreased proliferation by immobilized recombinant Thy-1 (Schmidt et al., 2015) as

Abbreviations: Fas, Fas receptor; FasL, Fas ligand; Wt, wild-type

Accepted manuscript published online 18 November 2015

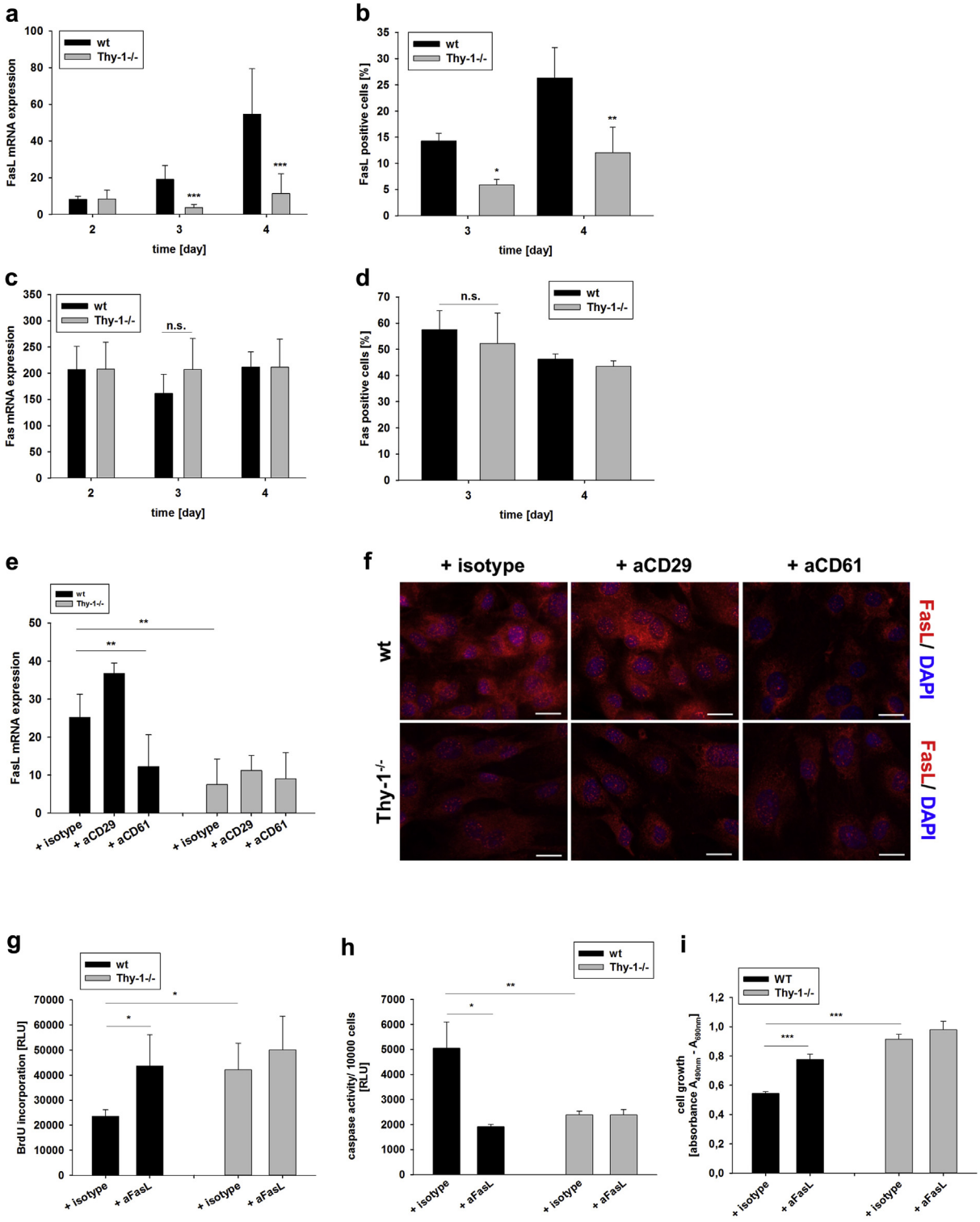

Figure 1. Thy-1–mediated apoptosis in dermal fibroblasts is mediated by up-regulation of FasL expression. Primary skin fibroblasts were enzymatically isolated from complete Thy-1–deficient (Thy-1$^{-/-}$) and wild-type (wt) control mice and analyzed for expression of Fas ligand (FasL) and Fas receptor. (**a**) FasL mRNA and (**b**) protein expression level of wt and Thy-1$^{-/-}$ fibroblasts at different time points analyzed by quantitative polymerase chain reaction and flow cytometry (fibroblasts of n ≥4 animals per genotype). (**c**) Fas receptor mRNA and (**d**) protein expression level of wt and Thy-1$^{-/-}$ fibroblasts (fibroblasts of n ≥4 animals per genotype). (**e, f**) Primary wt and Thy-1$^{-/-}$ fibroblasts were seeded for 72 hours in the presence or absence of function-blocking anti-CD61 (β3 integrin) or anti-CD29 (β1 integrin) antibody and appropriate isotype control. (**e**) FasL mRNA expression was detected by quantitative polymerase chain reaction (fibroblasts of n = 3 animals per genotype). (**f**) Representative images of FasL protein expression detected by immunofluorescence staining (red). Cell nuclei were counterstained with DAPI (blue). Bar = 50 μm. (**g–i**) Cell proliferation (after 24 hours), caspase 3/7 activity (after 72 hours), and cell growth (after 72 hours) of wt and Thy-1$^{-/-}$ fibroblasts in the presence or absence of function-blocking anti-FasL antibody and isotype control (fibroblasts of n = 4 animals per genotype). Data are given as mean ± SD. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$. BrdU, 5-bromo-2′-deoxyuridine; DAPI, 4′,6-diamidino-2-phenylindole; n.s., not significant; RLU, relative light units.