Identification of TROP2 (TACSTD2), an EpCAM-Like Molecule, as a Specific Marker for TGF-β1-Dependent Human Epidermal Langerhans Cells

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Langerin (CD207) expression is a hallmark of epidermal Langerhans cells (LCs); however, $CD207^+$ cells comprise several functional subsets. Murine studies showed that epidermal, but not dermal, $CD207^+$ cells require transforming growth factor-β 1 (TGF-β1) for development, whereas human data are lacking. Using gene profiling, we found that the surface molecule TROP2 (TACSTD2) is strongly and rapidly induced during TGF-β1-dependent LC commitment of human $CD34^+$ hematopoietic progenitor cells or monocytes. TROP2 is conserved between mouse and human, and shares substantial amino-acid identity with EpCAM, a marker for murine epidermal LCs. To our knowledge, neither TROP2 nor EpCAM expression has been analyzed in human dendritic cell (DC) subsets. We found that (i) all human epidermal LCs are $TROP2^+EpCAM^+$; (ii) human dermis lacks $CD207^+EpCAM^-$ or $CD207^+TROP2^-$ DCs, i.e., equivalents of murine dermal $CD207^+$ DCs; and (iii) pulmonary $CD207^+$ cells are $TROP2^-EpCAM^-$. Moreover, although EpCAM was broadly expressed by pulmonary and intestinal epithelial cells, as well as by bone marrow erythroid progenitor cells, these cells lacked TROP2. However, although TROP2 is expressed by human LCs as well as by human and murine keratinocytes, most murine LCs, except of a small subset, lacked TROP2. Therefore, TROP2 is a marker for human TGF-β1-dependent epidermal LCs.

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INTRODUCTION

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that can be found in a variety of lymphoid and non-lymphoid tissues (Merad and Manz, 2009). Although they are a promising target for vaccine development and tumor immunotherapy, their functional spectrum is not fully understood (Palucka *et al.*, 2011; Ueno *et al.*, 2011). Furthermore, most functional studies analyzing specific DC subsets are conducted in mice. In order to translate findings from murine models to the clinics, a better interspecies correlation of DC subsets is a prerequisite. Accordingly, human equivalents of murine DC subsets such as $CD8\alpha^+$ DCs resembling human $DNGR1^+$ DCs have recently been discovered (Bachem *et al.*, 2010; Crozat *et al.*, 2010; Jongbloed *et al.*, 2010; Poulin *et al.*, 2010). Considering increasing evidence for additional DC subsets identified in mouse (Bar-On *et al.*, 2010; Henri *et al.*, 2010), a better phenotypic analysis of human DC subsets is necessary.

Langerhans cells (LCs) are the DCs of the epidermis. In contrast to other DC sub-populations, they have traditionally been defined by their expression of the C-type Lectin receptor Langerin (CD207) (Romani *et al.*, 2010). With the recent discovery of $CD207^+$, dermal DCs, and the fact that CD207 is also expressed by $CD8\alpha^+$ DCs in lymphoid tissues in mice, the need for previously unreported specific LC markers became evident. The epithelial adhesion molecule EpCAM can be used to study murine skin DC subsets (Borkowski *et al.*, 1996b). Dermal and epidermal $CD207^+$ DCs can be discriminated by the expression of EpCAM (Bursch *et al.*, 2007; Ginhoux *et al.*, 2009; Nagao *et al.*, 2009a). However, despite these important observations in mice, data in the human system are still lacking.

In our study we aimed to identify previously unreported marker molecules for human epidermal LCs. Transforming growth factor-β 1 (TGF-β1) addition to cultures of monocytic cells is sufficient to induce an LC phenotype (Strobl *et al.*, 1996). Therefore, we screened for TGF-β1 response genes during LC lineage commitment using a genome-wide array approach.

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Abbreviations: DC, dendritic cell; LC, Langerhans cell; moDC, monocyte-derived dendritic cell; moLC, monocyte-derived Langerhans-like cell; TGF-β1, transforming growth factor-β 1

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We here identified TROP2, an evolutionary highly conserved molecule, which shows 48% amino-acid identity to EpCAM (El Sewedy *et al.*, 1998), as a specific marker for human epidermal LCs.

RESULTS

Identification of TROP2 as a TGF-β1 response gene during Langerhans cell commitment of CD34$^+$ cells

We recently undertook gene array profiling of purified human myeloid progenitor cells undergoing TGF-β1-dependent LC commitment and differentiation (data not shown). In this screen, serum-free cultures of flow-sorted myeloid-committed CD34$^+$CD45RA$^+$CD19$^-$ cord blood cells were supplemented with a combination of cytokines that promotes monocytic cell differentiation. Addition of TGF-β1 redirected monocyte to LC differentiation, as previously described by our group (Strobl *et al.*, 1997; Heinz *et al.*, 2006). Among the strongest induced genes at 6 and 24 hours after TGF-β1 stimulation was TACSTD2 (TROP2). Similar to EpCAM, TROP2 is expressed by epithelial tumor cells; in addition, flow-sorting experiments demonstrated that TROP2 expression allows to positively identify prostate stem cells and hepatic oval cells (Goldstein *et al.*, 2008; Okabe *et al.*, 2009). However, TROP2 expression by cells of the hematopoietic system was to our knowledge not described so far. Although TROP2 was strongly induced in response to TGF-β1 stimulation, EpCAM showed only weak induction (Figure 1a). Congruent with this, progenitor cells show much higher induction of TROP2 than EpCAM mRNA levels in TGF-β1-dependent day 7-generated LCs (Figure 1b). Protein expression analyses revealed that *in vitro*-generated CD207$^+$ LCs are TROP2$^+$EpCAM$^+$ (Figure 1c). Parallel cultures in which TGF-β1 had been omitted gave rise to monocytic cells and CD1a$^+$ cells lacking CD207 (Figure 1c lower panel and data not shown); these cells lacked detectable expression of TROP2 or EpCAM (Figure 1c, lower gates). LCs may gradually lose epithelial-associated marker molecules upon migration from epidermis to lymphoid tissues or during *in vitro* maturation, as previously demonstrated for E-cadherin (Jakob and Udey, 1998; Riedl *et al.*, 2000). Therefore, we analyzed whether LC activation/maturation is associated with changes in TROP2 or EpCAM expression. *In vitro*-generated CD34$^+$ progenitor cell-derived LCs were cluster purified as demonstrated previously (Gatti *et al.*, 2000), and subsequently induced to undergo maturation in response to tumor necrosis factor-α plus IL-1β stimulation. LC maturation was marked by strong upregulation of CD83 and CD86; average mean fluorescence intensities of TROP2 and EpCAM slightly

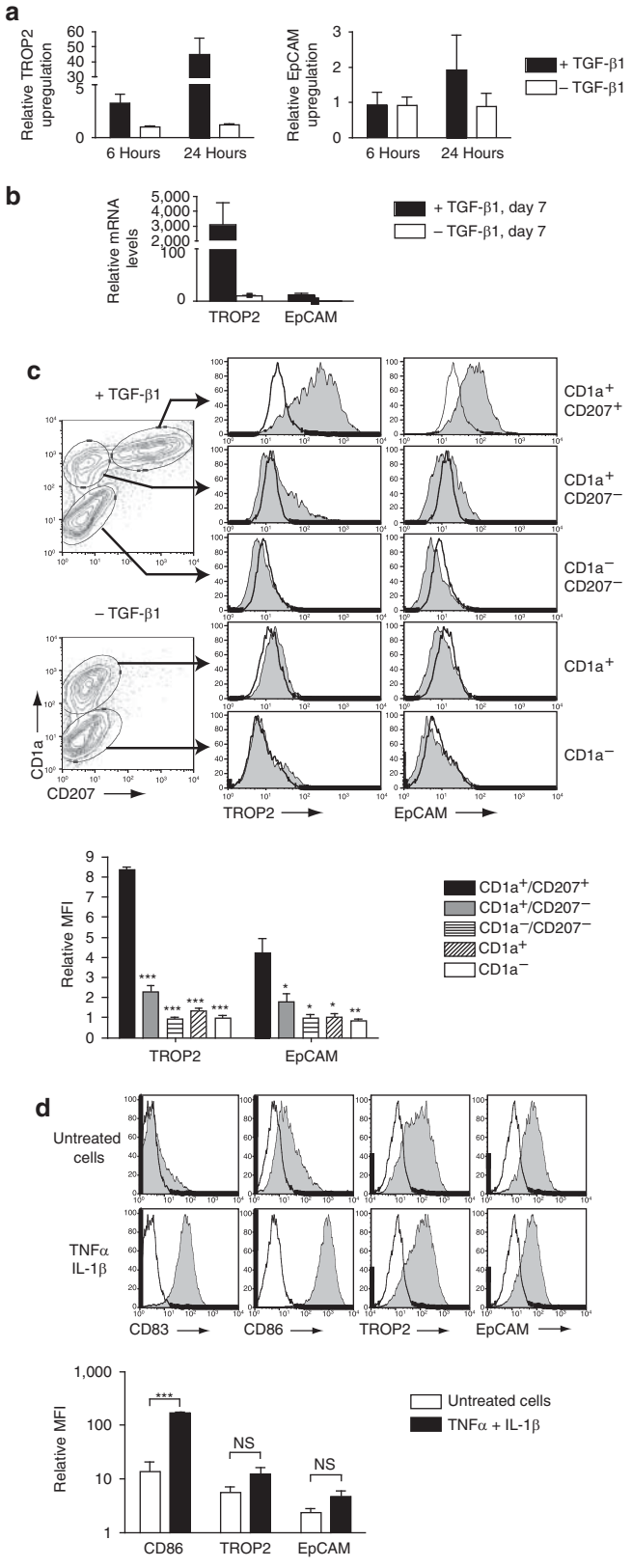

Figure 1. TROP2 is induced upon TGF-β1-dependent LC commitment of CD34$^+$ cells. (**a**) Bars represent mean upregulation of TROP2 and EpCAM at 6 and 24 hours relative to 0 hours after initiation of Langerhans cells (LC) cultures ± transforming growth factor (TGF)-β1 ($n = 6$ donors). (**b**) Real-time PCR analysis of TROP2 and EpCAM expression after 7 days of LC cultures ($n = 4$ donors). (**c**) Cells were gated after 7 days and were analyzed for TROP2 or EpCAM expression. (**d**) Day-7 LCs were cluster purified and stimulated with GM-CSF, tumor necrosis factor-α, and IL-1β for 48 hours or GM-CSF alone. Empty histograms in **c** and **d** represent isotype controls; bars in **b** and **c** represent the mean and SEM of three independent experiments. $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$. MFI, mean fluorescence intensity; NS, not significant.

increased during maturation (Figure 1d). We conclude that TROP2 is induced by TGF-β1 during LC commitment/differentiation of myeloid progenitor cells, and both TROP2 and EpCAM show stable expression during maturation of *in vitro*-generated CD207$^+$ LCs.

Erythropoietic cells express EpCAM but not TROP2

In order to evaluate TROP2 and EpCAM as potential LC-specific marker molecules, we systematically analyzed their expression among hematopoietic cells. Normal peripheral blood leukocytes lacked detectable expression of both markers (Figure 2a). Conversely, a minor subset of bone marrow cells expressed EpCAM but not TROP2 (Figure 2b). EpCAM and E-cadherin were previously found to be expressed by human erythropoietic cells (Lammers *et al.*, 2002). Congruent with these observations, most gated EpCAM$^+$ bone marrow cells represented glycophorin-A$^+$ erythroid progenitor cells (Figure 2b). As it is known that erythropoiesis transits through an EpCAM$^+$E-cadherin$^+$ stage and that TGF-β1 accelerates *in vitro* erythropoiesis (Zermati *et al.*, 2000), we analyzed whether TGF-β1 might induce TROP2 expression in erythropoietic progenitors. Thus, CD34$^+$ cord blood cells were induced to differentiate to erythropoietic cells in response to erythropoietin, IL-3, and stem cell factor in SFM. Parallel cultures were further supplemented with TGF-β1. FACS analyses confirmed previous observations (Zermati *et al.*, 2000): In the absence of TGF-β1, erythroid progenitors first acquired increasing CD71 expression; this was followed by the induction of glycophorin-A by CD71^{hi} cells (Figure 2c). TGF-β1 promoted the transition to glycophorin-A$^+$ cells (Figure 2c, left panel), with most cells positive for glycophorin-A and showing reduced CD71 expression (Figure 2c, lower panel). Although erythroid cells co-expressed E-cadherin and EpCAM as expected from a previous study (Lammers *et al.*, 2002), these cells lacked detectable TROP2 expression (Figure 2c). Moreover, TGF-β1 failed to induce TROP2 expression by erythropoietic cells (Figure 2c). These data demonstrate that TROP2 is not expressed by bone marrow erythropoietic cells, even when stimulated by TGF-β1, whereas erythropoiesis transits through an EpCAM$^+$E-cadherin$^+$ cell stage.

TROP2 is strongly expressed by moLCs, whereas these cells lack EpCAM

Previous studies revealed that the addition of TGF-β1 to GM-CSF plus IL-4-supplemented cultures of human monocytes induces the generation of DCs showing phenotypic characteristics of LCs (moLCs) (Geissmann *et al.*, 1998). To test whether moLC differentiation is accompanied by TROP2 and/or EpCAM expression, CD14$^+$ monocytes were induced to differentiate to classical monocyte-derived DCs (GM-CSF/IL-4) or moLCs (GM-CSF/IL-4/TGF-β1). Alternatively, they were cultured in GM-CSF only to induce macrophage differentiation. moLCs strongly expressed TROP2 but lacked detectable EpCAM (Figure 3). Additionally, a minor portion of monocyte-derived DCs in the absence of TGF-β1 weakly expressed TROP2. Furthermore, macrophages were TROP2$^-$EpCAM$^-$ (Figure 3). Therefore, moLCs generated in the presence of TGF-β1 strongly express TROP2, but lack detectable EpCAM expression.

TROP2 is expressed by epidermal Langerhans cells and keratinocytes *in situ*

Staining of epidermal-cell suspensions revealed that viable CD45$^+$HLADR$^+$CD207$^+$ LCs (identified in Figure 4a) are strongly positive for TROP2 and EpCAM (Figure 4b and c); CD45$^-$HLA-DR$^-$ keratinocytes (KCs) showed similar bright TROP2 staining as observed for LCs; however, these cells showed weaker expression of EpCAM compared with LCs (Figure 4a–c). *In situ* immunofluorescence analyses of TROP2 in healthy human breast skin revealed a strong epidermal staining pattern, thus confirming previous observations (Klein *et al.*, 1987). Co-staining with CD207 showed that LCs express TROP2 (Figure 4d). In comparison with TROP2, EpCAM showed weaker staining of KCs; however, CD207$^+$ cells co-expressed EpCAM to a similar extent as observed for TROP2 (Figure 4e). These data demonstrate that LCs *in situ* co-express TROP2 and EpCAM.

High levels of TROP2 expression on DCs in dermal preparations are restricted to migratory LCs

Dermal CD207$^+$ cells were recently identified in mice, based on the expression of CD207 in the absence of EpCAM. Whether human equivalents of murine dermal CD207$^+$ cells exist has, to our knowledge, not been studied. Human dermal DCs have previously been extensively studied leading to the delineation of at least three DC subsets (Klechevsky *et al.*, 2008). These include CD207$^+$CD1a^{hi} cells (Angel *et al.*, 2006), CD1a^{int} cells, as well as CD1a$^-$CD14$^+$ DCs. Although all three subsets could clearly be defined by flow cytometry (see gated populations in Figure 5a and data not shown), high levels of TROP2 and EpCAM expression were restricted to the small CD207$^+$CD1a^{hi} subset. The CD1a^{int} subset showed distinctively lower TROP2, and both markers were absent from the CD1a$^-$CD14$^+$ subset (Figure 5a, histograms and data not shown). Therefore, we demonstrate here that using a similar staining strategy as described for mice, human equivalents of murine dermal CD207$^+$ cells cannot be delineated.

DCs from lung and gut are TROP2$^-$/EpCAM$^-$

Similar to the epidermis, CD207$^+$ DCs have been described in several mucosal surfaces that are in contact with the environment, such as the lung, oral cavity, and vagina (Hladik *et al.*, 2007; Allam *et al.*, 2010; Van Pottelberge *et al.*, 2010). Conversely, the intestinal mucosa seems to be devoid of CD207$^+$ cells (Watanabe *et al.*, 2007). We screened lung and colon tissue for the presence of CD207$^+$TROP2$^+$EpCAM$^+$ cells. Although CD207$^+$ DCs could be detected within the pulmonary interstitial tissue, these cells lacked TROP2 and EpCAM expression; EpCAM, however, was expressed by the majority of the CD45$^-$ cells (Figure 5b, histograms). Intestinal lamina propria did not contain any detectable CD207$^+$ DCs (Figure 5c). The numerous HLA-DR$^+$ DCs within the lamina propria were similar to their pulmonary counterparts negative for TROP2

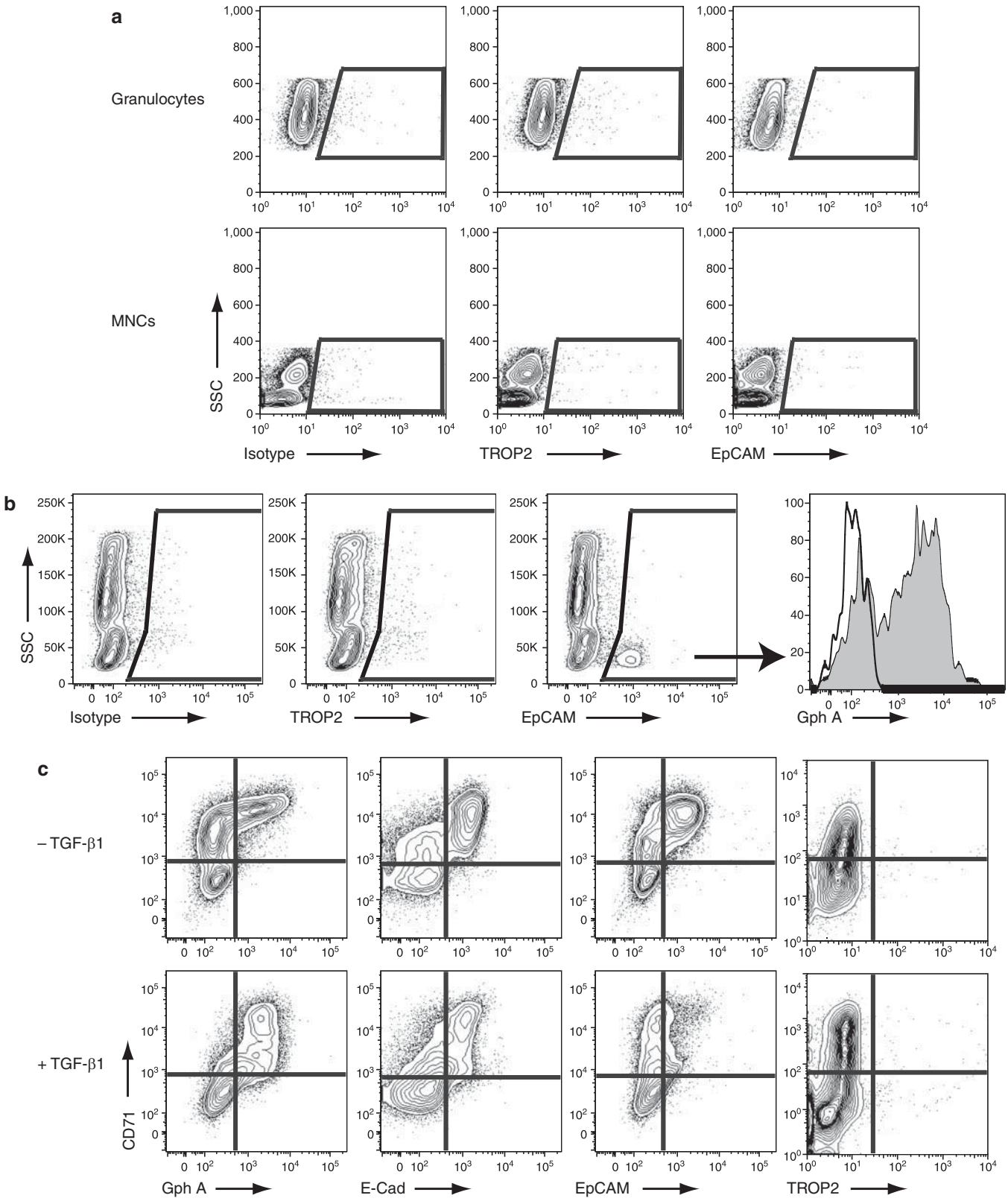

Figure 2. Lack of TROP2 but not EpCAM expression among erythropoietic cells. (**a**) FACS analysis of neutrophilic granulocytes and peripheral blood mononuclear cells (PBMCs) for TROP2 and EpCAM expression. (**b**) FACS analysis of a bone marrow aspirate for TROP2 and EpCAM expression. Empty histogram represents isotype control. (**c**) Hematopoietic progenitor cells were induced to differentiate *in vitro* from erythroid cells in response to erythropoietin, stem cell factor, and IL-3; parallel cultures were further supplemented with transforming growth factor (TGF)-β1. FACS analyses of cells after 5 days of culture. MNCs, mononuclear cells; SSC, side scatter.

and EpCAM, whereas the intestinal CD45$^-$ cells were EpCAM$^+$ (Figure 5c, histograms).

TROP2 allows the identification of a minor LC subfraction in mice

Human and murine TROP2 show 79% identical amino-acid composition (El Sewedy *et al.*, 1998). Although EpCAM expression within the murine skin has been extensively studied (Nagao *et al.*, 2009a), to our knowledge, TROP2 has not been analyzed so far. By examining epidermal ear sheets of wild-type C57BL/6 mice, we found TROP2 to be expressed by the majority of KCs, similar to what has been described in the human system (Klein *et al.*, 1987). However, most LCs (approximately 90%) lacked TROP2 (Figure 6a). Analyses of single-cell suspensions confirmed the histological data: Although KCs were all TROP2$^+$, the majority of LCs lacked

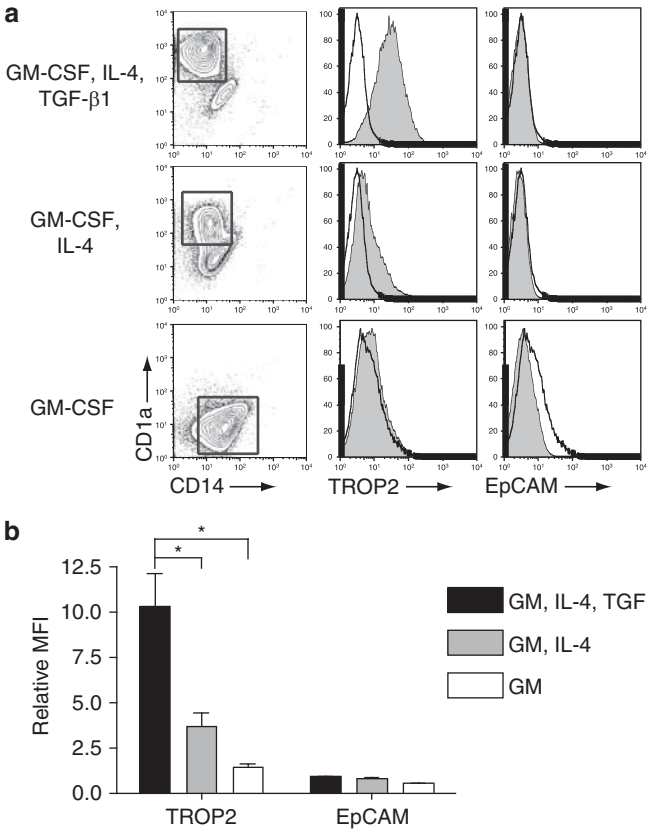

Figure 3. TROP2 is a marker for moLCs. (**a**) Surface expression of CD1a, CD14, TROP2, and EpCAM on the indicated dendritic cell (DC) subtypes after 5–7 days of culture. (**b**) Cells were gated as indicated and were analyzed for TROP2 and EpCAM expression. Empty histograms represent isotype controls. Bars represent the mean and SEM of three independent experiments. *$P<0.05$. GM-CSF, granulocyte macrophage colony-stimulating factor; IL-4, interleukin-4; MFI, mean fluorescence intensity; TGF-β1, transforming growth factor-β1.

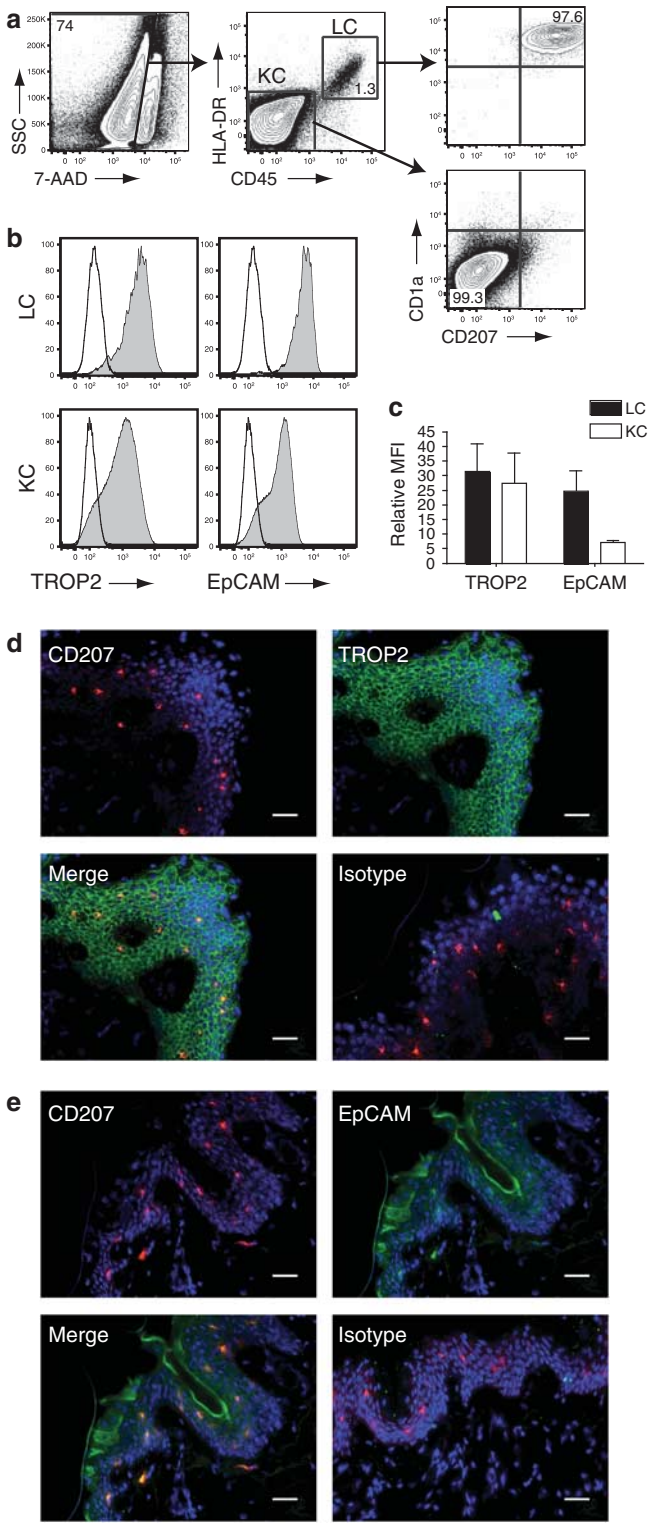

Figure 4. TROP2 and EpCAM expression by LCs and keratinocytes isolated from human skin and *in situ*. (**a**) FACS analysis of human epidermal single-cell suspensions. The gating strategy to identify LCs and keratinocytes (KC) is shown. (**b**) TROP2 and EpCAM expression by LC and KC as gated in **a**. Empty histograms represent isotype controls. One representative experiment of five is shown. (**c**) Relative mean fluorescence intensity (MFI) of TROP2 and EpCAM. Cells were analyzed as shown in **a** and **b**. Bars represent the mean and SEM of three independent experiments. (**d**, **e**) Tissue immunofluorescence staining of human breast skin cryosections for TROP2 (**d**) or EpCAM (**e**) and CD207. Nuclei are visualized with DAPI. Data are representative of four independent experiments. Bars = 25 μm. SSC, side scatter.

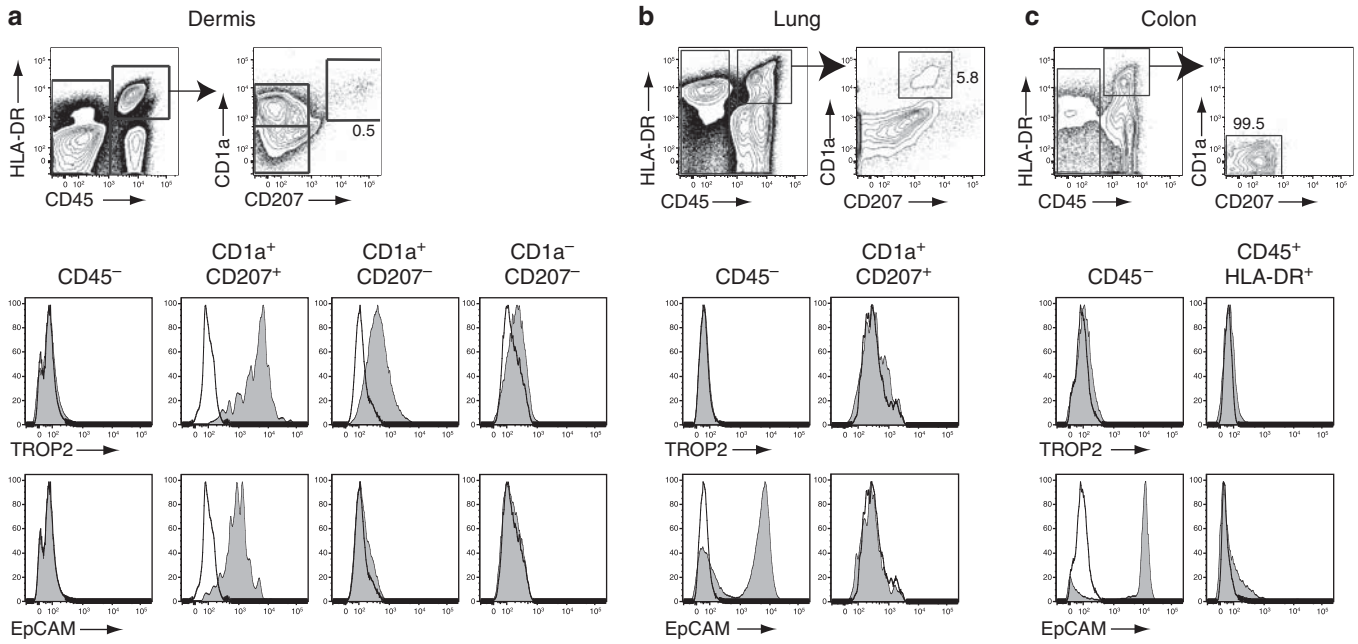

Figure 5. TROP2 and EpCAM are not expressed by human dermal-resident and interstitial DCs. FACS analysis of human dermal (**a**), lung (**b**), or colon (**c**) single-cell suspensions. 7-Aminoactinomycin D-negative cells were gated on a separate FACS diagram (not shown) and were analyzed for CD45 versus HLA-DR expression. $CD45^+HLA\text{-}DR^+$ cells were gated and were analyzed for CD207 versus CD1a expression; histograms represent phenotypically defined cell subsets analyzed for TROP2 or EpCAM; empty histograms represent isotype controls. Data are representative of $n=5$ (dermis), $n=4$ (lung), and $n=2$ (colon) experiments, each performed with a separate donor sample.

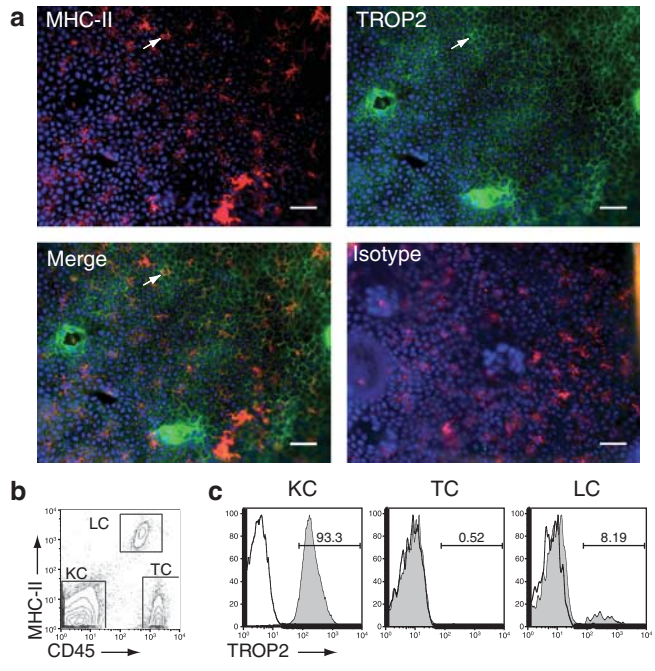

Figure 6. TROP2 expression in the murine epidermis. (**a**) Tissue immunofluorescence staining of murine epidermal ear sheet for TROP2 and major histocompatibility complex-II (MHC-II). Nuclei are visualized with DAPI. The arrowhead indicates a $TROP2^+MHC\text{-}II^+$ cell. Bars = 25 μm. (**b**) FACS analysis of a murine epidermal single-cell suspension. Gates were set to obtain cell fractions enriched for keratinocytes (KC, $CD45^-$), γδ-T-cells (TC, $CD45^+MHC\text{-}II^-$), and LC ($CD45^+MHC\text{-}II^+$). (**c**) TROP2 expression by sub-populations gated in **b**; empty histograms represent isotype controls; data are representative of 10 experiments. KC, keratinocytes; LC, Langerhans cells.

TROP2 expression. A minor fraction of $CD207^+$ cells were $TROP2^+$ (i.e., 9.8 ± 0.9%). Furthermore, epidermal γδ-T-cells, known to reside within $CD45^+MHC\text{-}II^-$ epidermal cell fractions, lack TROP2 (Figure 6b and c).

DISCUSSION

Anti-EpCAM stainings recently enabled the identification of a previously unreported murine DC subset, the so-called dermal $CD207^+$ DCs. EpCAM expression is restricted to epidermal LCs; therefore allowing to subdivide dermal $CD207^+$ cells into migratory epidermal LCs ($EpCAM^+$) and dermal-resident cells ($EpCAM^-$). To our knowledge, EpCAM has not been evaluated as a candidate marker molecule for the classification of human DC subsets.

Here we show that TROP2 and EpCAM are co-expressed by epidermal LCs. Moreover, we provide evidence that human dermis lacks an analogous population to murine dermal $CD207^+$ cells, and that $CD207^+$ DCs from lung differ from LCs in that they are mostly $TROP2^-EpCAM^-$; similarly intestinal DCs were $TROP2^-EpCAM^-$. We furthermore showed that TROP2, but not EpCAM, is strongly induced during TGF-β1-dependent LC lineage commitment of monocytopoietic cells. Finally, on the basis of TROP2 and EpCAM expression, TGF-β1-dependent $CD34^+$ cell-derived $CD1a^+CD207^+$ cells resemble LCs *in vivo*, therefore supporting other data that these cells are model cells of LCs.

Among the DC subsets analyzed in this study, only skin DCs expressed EpCAM and TROP2. These $EpCAM^+TROP2^+$ DCs represent epidermal-resident LCs as well as dermal-migratory LCs, the latter constituting a minor dermal

DC subset characterized by high expression levels of CD207 (Angel *et al.*, 2006). Although dermal-resident $CD1a^+CD207^-$ DCs were also $TROP2^+$, these cells clearly showed lower TROP2 expression levels compared with LCs. Furthermore, these cells lacked EpCAM. Similarly, moLCs generated in the presence of GM-CSF plus IL-4 and TGF-β1 were $TROP2^+EpCAM^-$. Therefore, among DC subsets, EpCAM might be a more specific marker for LCs than TROP2.

A key finding of our study is that all dermal $CD207^+$ DCs co-expressed EpCAM and TROP2. The lack of $CD207^+EpCAM^-$ cells in human dermis, therefore, implies that human equivalents to murine dermal $CD207^+$ DCs (previously identified as $CD207^+EpCAM^-$ dermal cells) do not exist. In this context, it is interesting that cells with this phenotype ($CD207^+EpCAM^-TROP2^-$) could readily be detected in lung. Our data therefore indicate that $CD207^+$ stainings in skin identify epidermal LCs, whereas $CD207^+$ lung DCs phenotypically differed from LCs in that these cells lacked EpCAM and TROP2. This study is to our knowledge the first to demonstrate that human pulmonary and epidermal $CD207^+$ cells show distinct phenotypic characteristics. These cells might represent human equivalents of murine $CD103^+CD11b^-CD207^+$ pulmonary DCs (Sung *et al.*, 2006), a possibility that might be tested using gene array profiling.

TROP2 mRNA is strongly induced within 24 hours during TGF-β1-dependent LC lineage commitment of myeloid progenitor cells. Accordingly, the addition of TGF-β1 to GM-CSF/IL-4-supplemented monocyte-derived DC cultures resulted in the induction of TROP2 along with the acquisition of other LC markers. TROP2 induction was associated with TGF-β1-dependent progenitor cell commitment to LCs and was not generally induced upon TGF-β1 stimulation of progenitors; e.g., TGF-β1 promoted erythroid progenitor cell differentiation, however, this effect was not accompanied by TROP2 expression. Therefore, TROP2 is a marker molecule for TGF-β1-dependent LCs. Future detailed studies should address the molecular mechanisms underlying TGF-β1-dependent TROP2 induction concomitant with LC differentiation.

We demonstrated that $CD34^+$ cell-derived TGF-β1-dependent $CD207^+$ cells are $EpCAM^+TROP2^+$; from these characteristics they resembled epidermal LCs. We previously demonstrated numerous cytoplasmic Birbeck granules in these cells, indistinguishable from *ex vivo* isolated LCs (Strobl *et al.*, 1996). Together, these findings support that these *in vitro*-generated DCs can be used as model cells for LCs. As mentioned above, moLCs expressed TROP2 but lacked EpCAM. Therefore, these moLCs lack certain hallmark features of LCs.

Our data on LC-associated TROP2 expression are compatible with previous observations that TGF-β1 is essential for the development of LCs (Strobl *et al.*, 1996; Borkowski *et al.*, 1996a; Kel *et al.*, 2010), whereas it is dispensable for the development of dermal DCs (Nagao *et al.*, 2009a; Kel *et al.*, 2010). Therefore, a bright TROP2 expression by LCs might be indicative for the requirement of LCs on constitutive-active TGF-β1 signaling. A positive relationship of TROP2 expression and TGF-β1 signaling may also exist for epithelial cells, as TROP2 allowed the selection of epithelial stem cells (Goldstein *et al.*, 2008) and TGF-β1 stimulated sphere formation of epithelial stem cell (Wang *et al.*, 2011).

It will be interesting to further study the small subset of murine $TROP2^+$ LCs. These cells might be enriched in proliferating LCs, in analogy to proliferating hepatic progenitors (Okabe *et al.*, 2009). Alternatively, $TROP2^+$ LCs might anatomically differ from $TROP2^-$ LCs. Interestingly, human epidermis is a multilayered tissue, with LCs residing in basal/suprabasal layers, whereas murine epidermis contains only a few layers. Furthermore, TROP2 expression is diminished in basal KC layers (Klein *et al.*, 1987), therefore showing a similar expression pattern as reported for TGF-β1 (Schuster *et al.*, 2009).

Finally, the question of the functional role of TROP2 and EpCAM in LC biology remains to be answered. EpCAM and TROP2 show non-redundant function in human as revealed by the genetic disorders gelantinous drop like conreal dystrophy, caused by TROP2 mutations (Tsujikawa *et al.*, 1999), and tufting enteropathy and Lynch syndrome, caused by EpCAM mutations (Sivagnanam *et al.*, 2008; Kempers *et al.*, 2011). Functional data in cancer cell lines indicate a pro-proliferative role for both molecules (Wang *et al.*, 2008; Cubas *et al.*, 2010). TROP2 may share downstream effector pathways with EpCAM; however, unlike EpCAM, the intracellular domain of TROP2 contains a conserved PIP_2 binding domain (El Sewedy *et al.*, 1998). The intracellular tail of EpCAM translocates to the nucleus to activate β-catenin signaling (Maetzel *et al.*, 2009), and EpCAM overexpression counter regulates E-cadherin adhesion (Winter *et al.*, 2003). As E-cadherin and β-catenin have been implicated in tolerogenic DC functions (Jiang *et al.*, 2007), it will be interesting to further study TROP2 and/or EpCAM function in *ex vivo*-generated LCs. Because a full knockout of EpCAM in mice is embryonically lethal (Nagao *et al.*, 2009b), DC-specific knockout models will be essential for *in vivo* studies.

With potential clinical applications of our study in mind, it is noteworthy that TROP2 expression was not detectable in bone marrow or peripheral blood. Together with the reported strong overexpression of TROP2 on metastatic tumor cells, studies should include anti-TROP2 stainings to detect and quantify circulating tumor cells; this strategy currently mostly relies on anti-EpCAM stainings (Criscitiello *et al.*, 2010).

MATERIALS AND METHODS

Cells and tissues

$CD34^+$ hematopoietic progenitor cells and $CD14^+$ monocytes were isolated from cord blood as described (Taschner *et al.*, 2007). Human and mouse epidermis and dermis were separated following overnight incubation on Dispase II (1.2 U ml^{-1}; Roche, Vienna, Austria) and incubated for 1 hour at 37 °C in phosphate-buffered saline containing DNAse I (200 μg ml^{-1}, Sigma-Aldrich, Vienna, Austria) and trypsin (2 mg ml^{-1}, Sigma-Aldrich) for epidermis or collagenase IV (4,000 U ml^{-1}, Sigma-Aldrich) for dermis. Single-cell suspensions were prepared by filtering the digested tissues through a 70-μm cell strainer (BD Biosciences, Schwechat, Austria). Single-cell suspensions of lung and intestinal tissue were prepared as described (Sauer *et al.*, 2006; Ng *et al.*, 2010). Lung and colon specimens were

obtained from individuals undergoing surgery for tumor removal. Samples were taken from unaffected tissue at the resection margin. All procedures were carried out in accordance to the guidelines from the Medical University of Vienna Institutional Review Board for these studies. Informed consent was provided in accordance with the Declaration of Helsinki Principles.

Cytokines and reagents

Human stem cell factor, thrombopoietin, tumor necrosis factor-α), GM-CSF, fms-related tyrosine kinase 3 ligand (FL), IL-1β, IL-3, and IL-4 were purchased from PeproTech (London, UK); TGF-β1 was purchased from R&D Systems (Wiesbaden, Germany); erythropoietin was purchased from Jannsen-Cilag (Vienna, Austria).

In vitro cell culture

$CD34^+$ cord blood cells were cultured serum-free for 2–3 days under progenitor expansion conditions (fms-related tyrosine kinase 3 ligand, stem cell factor, and thrombopoietin, each at 50 ng ml^{-1}) before subculturing with lineage-specific cytokines. LC cultures were described before (Strobl *et al.*, 1997). For some experiments, LC clusters were purified as described before (Gatti *et al.*, 2000) and then further cultured in the presence of GM-CSF (100 ng ml^{-1}), tumor necrosis factor-α (25 ng ml^{-1}), and IL-1β (20 ng ml^{-1}) to induce maturation. Erythroblast cultures and $CD14^+$ monocyte-derived DC, and moLC cultures were described before (Geissmann *et al.*, 1998; Zermati *et al.*, 2000).

Real-time PCR

Real-time PCR analysis was performed as previously described (Platzer *et al.*, 2009). The following primers were used: HPRT, forward 5′-GACCAGTCAACAGGGGACAT-3′, reverse 5′-AACACT TCGTGGGGTCCTTTTC-3′; EpCAM, forward 5′-TGCTCTGAGCGA GTGAGAA-3′, reverse 5′-TGCAGTCCGCAAACTTTTA-3′, and TR OP2, forward 5′-ACAACGATGGCCTCTACGAC-3′, reverse 5′-AGTT CACGCACCAGCACAC-3′.

Flow cytometry

Flow cytometry staining and analysis was performed as described (Platzer *et al.*, 2004). Mouse anti-human mAbs, specific for EpCAM (Alexa-Fluor 488-conjugated), CD324 (APC-conjugated), CD1a, and glycophorin A (Pacific Blue-conjugated), as well as rat anti-mouse mAbs specific for major histocompatibility complex-II (phycoerythrin (PE)-conjugated) and CD45 (APC/Cy7-conjugated) were purchased from Biolegend (Uithoorn, The Netherlands); mAbs specific for CD86 (PE-conjugated), HLA-DR (biotinylated), and CD83 (APC-conjugated), as well as streptavidin-PE/Cy7 were purchased from BD Biosciences; mAb specific for CD207 (PE-conjugated) was purchased from Immunotech (Marseille, France); mAb specific for CD45 (APC-conjugated) was purchased from E-Bioscience (Vienna, Austria); uncoupled mAb specific for CD71 (clone VIP1) was kindly provided by O. Majdic, Vienna, Austria; second step reagent was a polyclonal PE-conjugated goat anti-mouse antibody (Dako, Glostrup, Denmark); TROP2 was detected using a goat anti-human or anti-mouse uncoupled polyclonal antibody (R&D Systems); second step reagent was a polyclonal donkey anti-goat Alexa-Fluor 488-conjugated antibody (Invitrogen, Lofer, Austria). Cells were routinely stained with 7-aminoactinomycin D (Sigma-Aldrich) before analysis to exclude dead cells. Flow cytometric analysis was performed using an LSRII instrument (BD Biosciences) and the FlowJo software (Tree Star, Ashland, OR).

Immunohistology

Tissue specimens were stained as described (Göbel *et al.*, 2009). For detection of the TROP2 antibody, slides were probed with a polyclonal donkey-anti-goat Alexa-Fluor-488-conjugated antibody (Invitrogen). Nuclei were stained with DAPI and slides were mounted using fluorescent mounting medium (Dako). Pictures were taken using an Eclipse 80i microscope (Nikon, Amstelveen, The Netherlands) and Lucia G software (Laboratory Imaging, Prague, Czech Republic). Murine epidermal ear sheets were prepared as described (Nagao *et al.*, 2009a).

Statistical analysis

Statistical analysis was performed using the two-tailed Student's *t*-test; *P*-values of less than 0.05 were considered significant.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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