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<th>Antibody supplier &amp; catalogue number</th>
<th>Antibody research resource identifiers</th>
<th>Antibody species &amp; isotype</th>
<th>Antibody dilution</th>
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Table AF3.1. Targets for immunofluorescence (IF) labelling. Targets are listed together with common synonyms, HGNC symbols, UniProt identifiers, and antibody supplier details, research resource identifiers, species/isotype, and working dilution for the: mouse (Ms), rabbit (Rb), rat (Rt) and goat (Gt); monoclonal (mAb) and polyclonal (pAb) antibodies used in this study. Where relevant, the phosphorylated serine (pS), threonine (pT) and tyrosine (pY) residues are listed. Details of the secondary antibodies can be found in Table A2.3; please note that an anti-Ms IgG1 antibody was used as a secondary antibody for Keratin 14 (Ms IgG3 isotype) as it was found to perform better in preliminary experiments (Fig. AF3.1). Note that although pS218/pS222 is the canonical MEK1/2 dual phosphorylate site, as is pT185/pT187 for ERK1/2; pS338 is a surrogate marker for Raf-1 activity [1]. HGNC, HUGO gene nomenclature consortium; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase.
Mouse IgG3 anti-K14 showed a better signal with an anti-Ms IgG1 Alexa-488 secondary antibody. Human epidermis was labelled with a mouse IgG3 anti-keratin 14 antibody, prior to incubation with (A) an anti-mouse IgG1 Alexa-488 secondary antibody, and (B) an anti-mouse IgG3 Alexa-488 secondary antibody. The boundaries of the epidermis have been demarcated (dashed white lines) for reference, and images are oriented with the dermis to the left. Scale bars (at bottom right) represent 10 µm. Dermal and stratum corneum non-specific signal/autofluorescence is much higher for (B) than (A), as the gain and offset were increased to try detect the expected K14 signal.
### Regulation of integrin function

- **keratin 14 ⇔ integrin β4**
  - An indirect interaction between integrin β4 and K14, mediated by plectin, has been observed in MD-EB6 and PA-JEB immortalised keratinocytes [2].
  - Hemi-desmosomes containing integrin β4 have been observed to directly attach to the keratin cytoskeleton [3,4].
- **keratin 14 → integrin β4**
  - Keratins (including keratin 14) stabilise interactions between plectin and β4 integrin (limiting integrin β4 phosphorylation by phospho-ERK1/2) to maintain hemidesmosomes [5].
- **phospho-MEK1/2 =| integrin β4**
  - Transgenic expression of MEK1 (but not MEK2) has been shown to induce epidermal hyperplasia and increase expression of integrin β1, and this effect did not require an active MEK1 kinase domain [6].
- **MEK1/2 → integrin β1**
  - Within a transfected, immortalised mammary epithelial cell line (H82/Tn32) activation of c-erbB2 was shown to reduce α2β1 integrin mediated adhesion (through reduced activity, not reduced abundance), and this effect was blocked with the MEK1/2 inhibitor PD98059 [7].
  - Transgenic expression of MEK1 (but not MEK2) has been shown to induce epidermal hyperplasia and increase expression of integrin β4, and this effect did not require an active MEK1 kinase domain [6].
- **phospho-MEK1/2 =| integrin β1**
  - MEK1/2 mediated phosphorylation of ERK1/2 is a key step in the canonical Raf-MEK-ERK signalling cascade, and the signalling/reaction kinetics have been extensively parameterised in a number of cell lines including HeLa cells [17,18].
  - Interactions between Raf-1 and MEK1/2 occur as part of the canonical Raf-MEK-ERK signalling cascade, and the signalling/reaction kinetics have been extensively parameterised in a number of cell lines including HeLa cells [17,18].
  - MEK1/2 mediated phosphorylation of ERK1/2 is a canonical Raf-MEK-ERK signalling cascade interaction, and the signalling/reaction kinetics have been extensively parameterised in a number of cell lines including HeLa cells [17,18].
  - In primary-culture human keratinocytes, the addition of PD098059 (MEK1 inhibitor) has been shown to reduce phospho-ERK abundance and drive an associated reduction in the abundance of phosphorylated Elk-1 (a TF with a well-characterised response downstream of ERK1/2 signalling) [21].

### Regulation of MEK phosphorylation activity

- **14-3-3 σ ⇔ Raf-1**
  - Anti-stratifin (anti-14-3-3σ) chromatography was combined with liquid chromatography tandem mass spectrometry (LC-MS/MS) to examine the epithelial colon carcinoma cell line (DLD1-ETA cells); Raf-1 was detected indicating that it is a binding partner [8].
  - Raf-1 has also been shown to bind 14-3-3σ (stratifin) with a moderate affinity (in comparison to other Raf and 14-3-3 isoforms) [9].
- **14-3-3σ → phospho-ERK1/2**
  - The ectopic expression of stratifin within epithelial colon carcinoma cells (DLD1-ETA cell line) has been shown to increase phospho-ERK abundance using SDS-PAGE and WB with an anti-phospho-ERK1/2 antibody [10]; presumably this effect is mediated in part through the stratifin and Raf-1 interaction (see above).
- **calmodulin ↔ Raf-1**
  - Calmodulin has been shown to exert an inhibitory effect on ERK activation within keratinocytes, and it has been proposed that this effect helps to establish a signal-threshold for pathway activation [11].
- **keratin 10 =| phospho-ERK1/2**
  - The loss of keratin 10 has been shown to increase the abundance of phospho-ERK1/2 (and p38 MAPK) [12].
- **integrin β1 → phospho-ERK1/2**
  - Binding of α3β1 integrin to laminin-5 has been shown to induce MEK activation in an epithelial cell line [13] and mouse keratinocytes [14].
- **integrin β1 → phospho-ERK1/2**
  - Suprabasal ITGB1 expression is associated with increased phospho-ERK within the epidermis; furthermore, the incubation of cultured human keratinocytes with extracellular matrix ligands for integrin β1 has been shown to induce phospho-ERK [15]; presumably through phospho-MEK1/2 (see above).
- **integrin β4 → phospho-ERK1/2**
  - In HeLa cells (epithelial cervical adenocarcinoma), adhesion mediated by integrin β4 has been shown to increase c-Fos abundance; and this effect is dependent upon signalling through ERK1 and Rho activation [16].

### ERK-MAPK signal transduction

- **Raf-1 ⇔ phospho-Raf-1**
  - Phosphorylation of Raf-1 is a key step in the canonical Raf-MEK-ERK signalling cascade, and the signalling/reaction kinetics have been extensively parameterised in a number of cell lines including HeLa cells [17,18].
  - Interactions between Raf-1 and MEK1/2 occur as part of the canonical Raf-MEK-ERK signalling cascade, studied using a number of methods/cell lines including:
    - transactivated insect cell lines (S9), where it was shown that this interaction is not dependent upon MEK1 or Raf-1 kinase activity [19];
    - HEK293 (epithelial kidney cells), where c-Raf was detected in affinity pulldowns of tagged MEK1 [20];
    - HeLa cells, where the reaction kinetics have been parameterised [17].
- **phospho-Raf-1 → phospho-MEK1/2**
  - Raf-1 mediated phosphorylation of MEK1/2 is a key step in the canonical Raf-MEK-ERK signalling cascade interaction, and the signalling/reaction kinetics have been extensively parameterised in a number of cell lines including HeLa cells [17,18].
  - Interactions between MEK1/2 and ERK1/2 occur as part of the canonical Raf-MEK-ERK signalling cascade, and the signalling/reaction kinetics have been extensively parameterised in a number of cell lines including HeLa cells [17,18].
- **MEK1/2 → ERK1/2**
  - Phosphorylation of MEK1/2 is a key step in the canonical Raf-MEK-ERK signalling cascade, and the signalling/reaction kinetics have been extensively parameterised in a number of cell lines including HeLa cells [17,18].
  - MEK1/2 mediated phosphorylation of ERK1/2 is a canonical Raf-MEK-ERK signalling cascade interaction, and the signalling/reaction kinetics have been extensively parameterised in a number of cell lines including HeLa cells [17,18].

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ERK-MAPK downstream targets

- ERK has been shown to regulate activity of the transcription factor C/EBP (downstream of interferon signalling) [22].
- Within differentiating mouse epidermis it has been shown that expression of K10 is controlled by the C/EBP (and AP-2) transcription factors [23].

C/EBP \(\rightarrow\) K10

- The addition of PD98059 (MEK inhibitor) has been shown to block increases in c-Jun abundance which are induced by calcitriol (the hormonally active metabolite of vitamin D). This effect is presumably mediated by preventing phospho-MEK1/2 activation of phospho-JNK [24].
- Integrin β4 adhesion has been shown to increase c-Fos abundance in HeLa cells, and this effect is mediated by ERK1 and Rho activation [16].
- The hormonally active metabolite of vitamin D (calcitriol) has been shown to increase the abundance of c-Fos, and this is abrogated by the addition of the MEK1/2 inhibitor PD98059, suggesting the effects of calcitriol are mediated through phospho-MEK1/2 activation of phospho-ERK1/2 [24].

- The addition of epidermal growth factor to cultured human keratinocytes leads to an increase in the abundance of phosphorylated Fra-2, and these effects are abrogated by the addition of the MEK1/2 inhibitor PD98059, suggesting that Fra2 phosphorylation is mediated through MEK1/2:ERK1/2 signalling [25].
- The hormonally active metabolite of vitamin D (calcitriol) has been shown to increase the abundance of c-Fos, and this is abrogated by the addition of the MEK1/2 inhibitor PD98059, suggesting the effects of calcitriol are mediated through phospho-MEK1/2 activation of phospho-ERK1/2 [24].
- In DAB1 cells (urinary bladder carcinoma) it has been shown that Fra-2 binds to an AP-1 responsive element within the ITGB4 promoter region [28].

Table AF3.2 References for the ERK-MAPK-centric relationships/associations shown in Fig. 1. Experimental evidence supporting undirected (\(\cong\)), directed activating (\(\rightarrow\)) and directed inhibitory (\(==|\)) relationships. This is not a comprehensive list of all interactions or regulatory mechanisms active within the epidermis; rather it was used to motivate the selection of targets that may show regulatory changes along the gradient of keratinocyte differentiation. WB: western blotting; SDS-PAGE: SDS polyacrylamide gel electrophoresis; AP-1: Activator Protein-1; TF: transcription factor; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase.
References