

Keywords: I κ B α ; SUMO; Polycomb; Tumour Necrosis Factor (TNF α); Squamous Cell Carcinoma (SCC)

Novel functions of chromatin-bound I κ B α in oncogenic transformation

L Espinosa^{*,1}, A Bigas¹ and M C Mulero¹

¹Program in Cancer Research, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Doctor Aiguader, 88, Barcelona 08003, Spain

The nuclear factor- κ B (NF- κ B) signalling pathway participates in a multitude of biological processes, which imply the requirement of a complex and precise regulation. I κ B (for Inhibitor of kappaB) proteins, which bind and retain NF- κ B dimers in the cytoplasm, are the main contributors to negative regulation of NF- κ B under non-stimulation conditions. Nevertheless, increasing evidences indicate that I κ B proteins exert specific nuclear roles that directly contribute to the control of gene transcription. In particular, hypophosphorylated I κ B β can bind the promoter region of TNF α leading to persistent gene transcription in macrophages and contributing to the regulation of the inflammatory response. Recently, we demonstrated that phosphorylated and SUMOylated I κ B α reside in the nucleus of the cells where it binds to chromatin leading to specific transcriptional repression. Mechanistically, I κ B α associates and regulates Polycomb Repressor Complex activity, a function that is evolutionary conserved from flies to mammals, as indicate the homeotic phenotype of *Drosophila* mutants. Here we discuss the implications of chromatin-bound I κ B α function in the context of tumorigenesis.

The nuclear factor- κ B (NF- κ B) family of transcription factors has an important role in the regulation of biological processes such as immunity and inflammation, apoptosis, stress response or ageing (Hayden and Ghosh, 2004). This family is composed of five members: RelA (p65), RelB and c-Rel, and the precursor proteins NF- κ B1 (p105) and NF- κ B2 (p100) that can be processed into p50 and p52, respectively. Nuclear factor- κ B transcription factors function as homo- and hetero-dimers that activate or repress gene expression in a context-dependent manner. Under resting conditions, most NF- κ B resides in the cytoplasm bound to particular inhibitory proteins of the inhibitor of kappaB (I κ B) family, I κ B α , I κ B β or I κ B ϵ , which generate an additional degree of complexity. Phosphorylation of I κ B at specific serine residues by the I κ B kinase (IKK) complex induces its ubiquitylation and subsequent proteasomal degradation, thus releasing the NF- κ B factor leading to its nuclear translocation and gene transcription (Beg *et al*, 1992). However, recent studies have provided evidence that I κ B proteins are more than NF- κ B inhibitors. In 2010, Rao and colleagues demonstrated that following LPS stimulation, hypophosphorylated newly synthesised I κ B β binds p65 and c-Rel at the promoter region of specific genes such as TNF α and protects the NF- κ B factor from I κ B α association and chromatin release leading to persistent transcriptional activity (Rao *et al*, 2010). The

same mechanism operates at the IL1- β promoter (Scheibel *et al*, 2010).

Some years ago, we found that in fibroblasts nuclear I κ B α associates with NF- κ B-independent gene promoters, such as *hes1* and *herp2*, which are general regulators of cell differentiation, correlating with their transcriptional repression. TNF α treatment induced IKK recruitment to these particular genes leading to I κ B α dissociation and transcriptional activation (Aguilera *et al*, 2004). Importantly, IKK was found constitutively bound to *hes1* and *herp2* promoters in colorectal cancer cells associated with increased gene expression (Fernandez-Majada *et al*, 2007a). Now, we have demonstrated that in keratinocytes there is a fraction of phosphorylated and SUMOylated I κ B α (PS-I κ B α) that binds to chromatin through direct association with histones leading to NF- κ B-independent gene regulation (Mulero *et al*, 2013). Interestingly, nuclear PS-I κ B α seems to be crucial for proper skin homeostasis, which might explain the severe skin phenotype of I κ B α -deficient mice that die 5 days after birth because of a massive skin inflammation likely associated with a defective barrier function (Beg *et al*, 1995; Klement *et al*, 1996; Rebholz *et al*, 2007; Table 1). In addition, our results strongly suggest that aberrant accumulation of cytoplasmic I κ B α in keratinocytes promotes squamous cell carcinoma (SCC;

*Correspondence: Dr L Espinosa; E-mail: lespinosa@imim.es

Received 28 August 2013; revised 19 November 2013; accepted 24 January 2014; published online 18 September 2014

© 2014 Cancer Research UK. All rights reserved 0007–0920/14



Table 1. Skin phenotypes associated to I κ B α mutant mouse models

Model	Phenotype	Reference
Conventional I κ B α -deficient mice	Increase on proliferative keratinocytes relative to differentiated cell types accompanied by a widespread dermatitis. Skin inflammation associated to high levels of IL-1 β and IFN- γ in the dermis and infiltration of CD8+ T cells and Gr-1+ neutrophils in the epidermis	Beg <i>et al</i> , 1995; Klement <i>et al</i> , 1996; Rebholz <i>et al</i> , 2007
Keratin5 promoter- I κ B α -deficient mice	Abnormal proliferation of keratinocytes without epidermal inflammation	Rebholz <i>et al</i> , 2007
Double I κ B α - and TNF α -deficient mice	Viable more than 3 months. Skin phenotype rescued	Shih <i>et al</i> , 2009
Keratin5 promoter- I κ B α -SR transgenic	Severe macroscopic phenotype characterised by flaky skin, hair loss, dysplasia of the epidermis and development of SCC and inflammatory response	van Hogerlinden <i>et al</i> , 1999; Seitz <i>et al</i> , 1998; van Hogerlinden <i>et al</i> , 2004
Human keratinocytes expressing I κ B α -SR and/or RAS Gly12Val transplanted into CB-17 scid mice	I κ B α -SR alone showed mild hyperplasia. Co-expression of RAS and I κ B α -SR produced large neoplasms resembling SCC	Dajee <i>et al</i> , 2003
Nfkbia ^{NES/NES} mice harbouring a triple point mutation in the NES	Defect on secondary lymphoid organ formation and impaired B-cell maturation. Expansion of the proliferative compartment and reduction of the differentiation layers in the skin	Wuerzberger-Davis <i>et al</i> , 2011; Mulero <i>et al</i> , 2013

Abbreviations: I κ B = inhibitor of kappaB; IL = interleukin; INF = interferon; NES = nuclear export sequence; SCC = squamous cell carcinoma; SR = super repressor.

Mulero *et al*, 2013), which is in agreement with the oncogenic phenotype of ectopic I κ B α -SR expression in the skin (van Hogerlinden *et al*, 1999, 2004).

I κ B α , AN OLD CYTOPLASMIC PROTEIN WITH A NOVEL NUCLEAR FUNCTION, OR VICE-VERSA

I κ B α regulates epidermal homeostasis. Mammalian epidermis consists of four different layers: basal, spinous, granular and cornified. The proliferating basal layer of the epidermis contains most of the stem and progenitor cells of this tissue, which then undergoes a differentiation process that culminates in their fully maturation and enucleation as they reach the surface layer, thus generating the cornified layer of keratinocytes. However, this is not an easy task for a tissue that is permanently exposed to a plethora of external insults such as UV radiation, extreme temperature variations and chemical exposure among others. The success of the skin differentiation process depends on the integration of intrinsic cellular programmes with external signals including the response of the immune system. Recently, we found that PS-I κ B α is predominantly distributed in the nucleus of keratinocytes bound to the promoter of genes such as HOX and IRX (Mulero *et al*, 2013), which control skin differentiation and proliferation but are also involved in the developmental regulation. Once in the chromatin, PS-I κ B α facilitates the recruitment of polycomb repressive complex 2 to gene promoters and dictates their competence to be induced following TNF α stimulation, thus establishing a mechanistic link between inflammatory signals and skin homeostasis. Remarkably, this role for I κ B α as a regulator of polycomb function is operating during *Drosophila* development since mutations in Cactus (the I κ B α orthologue in flies) enhanced the homeotic phenotype of Pc (Polycomb) mutations. These results together with the identification of I κ B α orthologues in the worm *Caenorhabditis elegans* that does not have a real NF- κ B pathway suggest that this newly identified function of PS-I κ B α is an ancestral function previous to the specialisation of the immune system.

Nuclear I κ B α as a tumour suppressor in the skin. Indicative of a tumour-suppressor function for PS-I κ B α , immunohistochemical

analysis of a panel of human skin samples demonstrated that I κ B α remains nuclear in the keratinocytes of benign skin lesions such as elastosis, psoriasis, actinic keratosis and Bowen disease, but is specifically lost in the more malignant lesions such as SCC. Further analysis of a cohort of 112 patients with urogenital SCC at different stages of tumour progression showed that in samples corresponding to invasive and metastatic SCC, I κ B α was totally excluded from the nucleus but accumulated in the cytoplasm (Mulero *et al*, 2013), suggesting that beyond loss of nuclear I κ B α , cytoplasmic I κ B α (likely SUMOylated-I κ B α) might have additional pro-tumorigenic functions, which will be investigated. This possibility is in agreement with the skin phenotype of different mice strains ectopically expressing the I κ B α super-repressor mutant (I κ B α -SR), which develop SCC-resembling tumours in the skin (Dajee *et al*, 2003; van Hogerlinden *et al*, 1999, 2004; Table 1), what contrast with the dogma that constitutive NF- κ B activation results in pro-tumorigenic phenotypes that are prevented following NF- κ B inhibition (Ben-Neriah and Karin, 2011). Similarly, Dajee *et al* (2003) demonstrated that mutant RASV12 failed to generate tumours when expressed in primary human keratinocytes (that were growth arrested), but it efficiently induced tumours resembling human SCC when co-expressed with I κ B α -SR. Based on others and our results, we speculate that cytoplasmic accumulation of I κ B α in skin tumours might sequester nuclear co-repressors and HDACs (Aguilera *et al*, 2004; Fernandez-Majada *et al*, 2007a, 2007b; Hoberg *et al*, 2004), thus promoting a global miss-regulation of gene transcription. In this sense, specific HDAC proteins contain SUMO-binding elements that could favour their binding to PS-I κ B α either in the nucleus or the cytoplasm (model in Figure 1). Whether the cytoplasmic I κ B α that is found in SCC samples is SUMOylated and/or phosphorylated remains to be addressed.

More recently, a knock-in mouse containing an I κ B α protein with a mutated nuclear export sequence that is mostly localised in the nucleus of most cells has been characterised. These mutant mice show impaired canonical and alternative NF- κ B pathways in the mature B cells with the absence of secondary lymphoid organs, but they display functional skin with no evidence of critical barrier defects (Wuerzberger-Davis *et al*, 2011). However, a detailed analysis of mutant skins from 7- to 8-week-old animals revealed significant

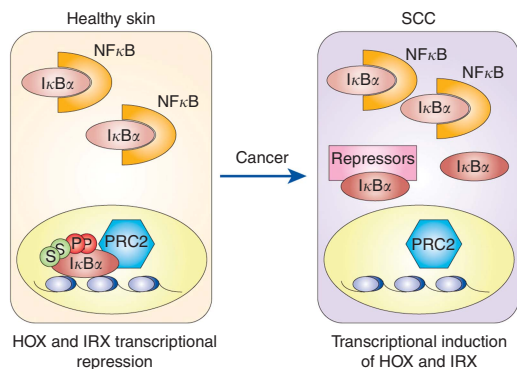


Figure 1. Schematic representation of PS-I κ B α structure depicting the six ankyrin repeats and the C-terminal PEST domain. SUMOylable lysines K21 and K22 are shown in green, whereas serines 32 and 36 (that are phosphorylated in PS-I κ B α) are indicated in red. Nuclear I κ B α is aberrantly localised in the cytoplasm of skin cancer cells. Thus, whereas in healthy skin, PS-I κ B α binds chromatin at the promoter of HOX and IRX family of genes repressing their transcription, in SCC, I κ B α is excluded from the nucleus and accumulates in the cytoplasm of the cells likely sequestering specific transcriptional repressors.

differences with wild-type mice including increased proliferation index, expansion of the K14-positive basal layer, and reduction on the thickness of the intermediate K10-positive skin layer (Mulero *et al*, 2013). Future experiments should address the question of whether constitutively nuclear I κ B α results in altered *Hox* and *Irx* expression in the basal or supra-basal layer of keratinocytes, and whether it protects these cells from undergoing tumorigenesis under specific transformation procedures or even with ageing.

I κ B kinase alpha (IKK α) modulates I κ B α localisation in keratinocytes. It is worth mentioning, that not only I κ B α but also the IKK α displays a predominantly nuclear distribution in the skin (Marinari *et al*, 2008; Zhu *et al*, 2007). Loss of IKK α associates with altered proliferation and differentiation of epidermal keratinocytes (Hu *et al*, 1999; Sil *et al*, 2004), whereas mutations that resulted in IKK α loss or in the generation of truncated proteins that failed to interact with chromatin led to the development of skin papillomas and SCC (Liu *et al*, 2006; Park *et al*, 2007). Conversely, chemically induced skin carcinogenesis is reverted by overexpression of wild-type IKK α (Liu *et al*, 2006). Our recent results indicated that active IKK α induced a partial accumulation of SUMOylated-I κ B α in the cytoplasmic compartment of keratinocytes, and IKK activation correlates with cytoplasmic accumulation of I κ B α in human SCC samples (Mulero *et al*, 2013). These results open the possibility that I κ B α miss-regulation might contribute to the phenotypes observed downstream of IKK α alterations, although it remains to be studied how IKK α regulates I κ B α distribution. Because chromatin-bound I κ B α is already phosphorylated in the residues known to be targets of canonical IKK activity, and we found that this phosphorylation is IKK independent, we speculate that nuclear IKK α might induce PS-I κ B α chromatin release by regulating specific editing enzymes (i.e., phosphatase and desumoylase), scaffold proteins or proteins associated with the repressive PS-I κ B α complex such as histones or members of the polycomb repressive complex 2, resulting in a reduced affinity of I κ B α with chromatin.

Different I κ B α populations include different post-translational modifications. Several publications previously established the link between SUMOylation and transcriptional repression by specific transcription factors such as CtBP, KAP1 or Sp3 (reviewed in Garcia-Dominguez and Reyes (2009)). SUMOylated-I κ B α had

previously been identified in different types of cells, however, no specific functions for this modified version of the molecule had been ascribed. In 1998, Desterro and colleagues detected the existence of endogenous SUMO-1-modified I κ B α in a panel of cancer cell lines, being SUMO-1 linked to lysine 21 of I κ B α . Because lysine 21 is involved in the signal-mediated degradation of I κ B α (following poly-ubiquitination), SUMO1-I κ B α was resistant to degradation and led to reduced NF- κ B activity in these cells. Interestingly, SUMOylation was reduced in I κ B α mutant proteins mimicking serine 32 and 36 phosphorylation that is a mark for degradation (Desterro *et al*, 1998). Accumulation of SUMO-1-bound I κ B α is also detected downstream of adenosine signalling during hypoxia and reoxygenation (Liu *et al*, 2009), although its functional contribution in this system is unknown. Recently, it was shown that I κ B α was modified by SUMO-2/3 but also by a combination of SUMO and ubiquitin chains (Culver *et al*, 2010) resulting in an I κ B α molecule that can still be regulated by degradation following NF- κ B activation (Aillet *et al*, 2012). The use of particular cell types, and the variations in the technical approaches (including the reagents used for stabilising SUMO chains and for detection) can explain the different results obtained by each group, however, it is still possible that several modifications coexist in particular I κ B α subpopulations leading to functional specificities. Interestingly, SUMOylable proteins are characterised by the presence of a specific Ψ KxE/D motif, which is the case of I κ B α but not of other I κ B orthologues such as I κ B β (Kracklauer and Schmidt, 2003). An extended consensus has also been reported, in which SUMOylation is imposed by negatively charged residues (including phosphorylated aminoacids) downstream of the Ψ KxE/D motif (Hietakangas *et al*, 2003). Thus, one could speculate that I κ B α SUMOylation is subsequent to its phosphorylation at serines 32 and/or 36 by specific kinase/s other than IKK β (Mulero *et al*, 2013). Thus, it is of crucial importance to identify such kinases, investigate how they are regulated under specific conditions, different tissues or particular processes, and evaluate their contribution to I κ B α SUMOylation and function. Finally, studying the contribution of SUMO modifications to I κ B α function is not an easy job as mutants that fail to be SUMOylated (i.e., the K21-22 mutant) are also unable to be ubiquitinated and degraded, thus leading to strong NF- κ B phenotypes. To circumvent this problem, we are currently generating SUMO-I κ B α fusion proteins, which will be tested for their chromatin- or NF- κ B-related activities.

The discovery that PS-I κ B α exerts a nuclear function that affects NF- κ B-independent transcription in response to NF- κ B-related stimuli such as TNF α is also a temptation to re-examine previous results that might have misinterpreted because of the most prominent role of cytoplasmic I κ B α . A putative involvement of PS-I κ B α in other types of cancer should also be studied; mainly in those that are associated with aberrant or chronic IKK activity such is the case of inflammation-related ones.

On the other hand, it is possible that specific elements of the chromatin-bound I κ B α complex are directly involved in I κ B α SUMOylation. For example, Pc2, one of the mammalian orthologues of the *Drosophila* Polycomb (PRC1) that we found as functionally associated with I κ B α /Cactus during *Drosophila* development, is a SUMO E3 ligase (Kagey *et al*, 2003). Moreover, it is not just I κ B α but other chromatin components such as the histones and elements of the PRC complex including EZH2 and SUZ12 are capable to be SUMOylated, which might impact on their capacity to recruit transcriptional repressors at specific genomic regions, which will be addressed in a near future.

Future therapeutic perspectives based on I κ B α . The importance of investigating the mechanisms involved in I κ B α and PS-I κ B α regulation and their functional relevance in tumour progression is evidenced by the fact that over 250 000 patients are diagnosed with

SCC in the world each year. Squamous cell carcinoma is the second most common skin cancer that arises in any region of the body but more frequently in areas exposed to the UV light. Although infrequent (about 10% of the cases), the appearance of metastasis in SCC patients has a very bad prognosis because the absence of treatments, especially in aged patients. Moreover, to date there are no good biomarkers that predict metastatic SCC potential beyond the previous appearance of local relapse, tumour staging or the presence of MYC amplifications (Toll *et al*, 2009). We are currently investigating whether detection of cytoplasmic I κ B α or PS-I κ B α , or/and nuclear-active IKK α identifies the small fraction of SCC patients that will develop tumour relapse or metastasis. Those patients could be then selected for more aggressive therapies, but also as candidates for future personalised treatments based on I κ B α /IKK α -based therapies.

CONCLUSIONS

Initially, I κ B proteins were described as cytoplasmic regulators of the NF- κ B pathway; however, several studies focused on I κ B α and I κ B β demonstrated the existence of specific nuclear roles for both proteins. First, it was found that I κ B α contains a functional nuclear localisation signal that is essential for the efficient post-activation termination of NF- κ B signalling by facilitating chromatin release of p50-p65 dimers (Arenzana-Seisdedos *et al*, 1997). However, I κ B α can be retained bound to the chromatin at specific promoter regions through its interactions with histones H2A and H4, thus playing an essential contribution to skin differentiation. We propose that PS-I κ B α can exert both pro-oncogenic and tumour-suppressor functions, which need to be investigated in detail. In contrast, nuclear I κ B β is mainly associated with the regulation of the inflammatory response, through the maintenance of persistent cytokine expression in macrophages. The finding that this polycomb-associated I κ B α function is conserved in the *Drosophila* development, together with and the identification of I κ B homologues in worms lacking a truly NF- κ B pathway suggests that this is an ancestral function that might exert a more general contribution to the cellular physiology. Finally, the identification of specific post-translational modifications that are restricted to chromatin-bound I κ B α converts its modifying enzymes and PS-I κ B α in attractive targets for novel therapeutic strategies specific for particular I κ B α -associated pathologies and patients.

ACKNOWLEDGEMENTS

LE is an investigator at the Carlos III program. MM is an investigator of the Sara Borrell program (CD09/00421). This work was supported by Instituto de Salud Carlos III Grant PI13/00448 to LE, Red Temática de Investigación Cooperativa en Cáncer (RD12/0036/0054) and Agència de Gestió d'Ajuts Universitaris i de Recerca (2009SGR-23).

REFERENCES

- Aguilera C, Hoya-Arias R, Haegeman G, Espinosa L, Bigas A (2004) Recruitment of IkappaBalpha to the hes1 promoter is associated with transcriptional repression. *Proc Natl Acad Sci USA* **101**(47): 16537–16542.
- Aillet F, Lopitz-Otsoa F, Egana I, Hjerpe R, Fraser P, Hay RT, Rodriguez MS, Lang V (2012) Heterologous SUMO-2/3-ubiquitin chains optimize IkappaBalpha degradation and NF-kappaB activity. *PLoS One* **7**(12): e51672.
- Arenzana-Seisdedos F, Turpin P, Rodriguez M, Thomas D, Hay RT, Virelizier JL, Dargemont C (1997) Nuclear localization of I kappa B alpha promotes active transport of NF-kappa B from the nucleus to the cytoplasm. *J Cell Sci* **110**(Pt 3): 369–378.
- Beg AA, Ruben SM, Scheinman RI, Haskill S, Rosen CA, Baldwin AS Jr (1992) I kappa B interacts with the nuclear localization sequences of the subunits of NF-kappa B: a mechanism for cytoplasmic retention. *Genes Dev* **6**(10): 1899–1913.
- Beg AA, Sha WC, Bronson RT, Baltimore D (1995) Constitutive NF-kappa B activation, enhanced granulopoiesis, and neonatal lethality in I kappa B alpha-deficient mice. *Genes Dev* **9**(22): 2736–2746.
- Ben-Neriah Y, Karin M (2011) Inflammation meets cancer, with NF-kappaB as the matchmaker. *Nat Immunol* **12**(8): 715–723.
- Culver C, Sundqvist A, Mudie S, Melvin A, Xirodimas D, Rocha S (2010) Mechanism of hypoxia-induced NF-kappaB. *Mol Cell Biol* **30**(20): 4901–4921.
- Dajee M, Lazarov M, Zhang JY, Cai T, Green CL, Russell AJ, Marinkovich MP, Tao S, Lin Q, Kubo Y, Khavari PA (2003) NF-kappaB blockade and oncogenic Ras trigger invasive human epidermal neoplasia. *Nature* **421**(6923): 639–643.
- Desterro JM, Rodriguez MS, Hay RT (1998) SUMO-1 modification of IkappaBalpha inhibits NF-kappaB activation. *Mol Cell* **2**(2): 233–239.
- Fernandez-Majada V, Aguilera C, Villanueva A, Vilardell F, Robert-Moreno A, Aytes A, Real FX, Capella G, Mayo MW, Espinosa L, Bigas A (2007a) Nuclear IKK activity leads to dysregulated notch-dependent gene expression in colorectal cancer. *Proc Natl Acad Sci USA* **104**(1): 276–281.
- Fernandez-Majada V, Pujadas J, Vilardell F, Capella G, Mayo MW, Bigas A, Espinosa L (2007b) Aberrant cytoplasmic localization of N-CoR in colorectal tumors. *Cell Cycle* **6**(14): 1748–1752.
- Garcia-Dominguez M, Reyes JC (2009) SUMO association with repressor complexes, emerging routes for transcriptional control. *Biochim Biophys Acta* **1789**(6–8): 451–459.
- Hayden MS, Ghosh S (2004) Signaling to NF-kappaB. *Genes Dev* **18**(18): 2195–2224.
- Hietakangas V, Ahlskog JK, Jakobsson AM, Hellesuo M, Sahlberg NM, Holmberg CI, Mikhailov A, Palvimo JJ, Pirkkala L, Sistonen L (2003) Phosphorylation of serine 303 is a prerequisite for the stress-inducible SUMO modification of heat shock factor 1. *Mol Cell Biol* **23**(8): 2953–2968.
- Hoberg JE, Yeung F, Mayo MW (2004) SMRT derepression by the IkappaB kinase alpha: a prerequisite to NF-kappaB transcription and survival. *Mol Cell* **16**(2): 245–255.
- Hu Y, Baud V, Delhase M, Zhang P, Deerinck T, Ellisman M, Johnson R, Karin M (1999) Abnormal morphogenesis but intact IKK activation in mice lacking the IKKalpha subunit of IkappaB kinase. *Science* **284**(5412): 316–320.
- Kagey MH, Melhuish TA, Wotton D (2003) The polycomb protein Pc2 is a SUMO E3. *Cell* **113**(1): 127–137.
- Klement JF, Rice NR, Car BD, Abbondanzo SJ, Powers GD, Bhatt PH, Chen CH, Rosen CA, Stewart CL (1996) IkappaBalpha deficiency results in a sustained NF-kappaB response and severe widespread dermatitis in mice. *Mol Cell Biol* **16**(5): 2341–2349.
- Kracklauer MP, Schmidt C (2003) At the crossroads of SUMO and NF-kappaB. *Mol Cancer* **2**: 39.
- Liu B, Park E, Zhu F, Bustos T, Liu J, Shen J, Fischer SM, Hu Y (2006) A critical role for I kappaB kinase alpha in the development of human and mouse squamous cell carcinomas. *Proc Natl Acad Sci USA* **103**(46): 17202–17207.
- Liu Q, Li J, Khoury J, Colgan SP, Ibla JC (2009) Adenosine signaling mediates SUMO-1 modification of IkappaBalpha during hypoxia and reoxygenation. *J Biol Chem* **284**(20): 13686–13695.
- Marinari B, Moretti F, Botti E, Giustizieri ML, Descargues P, Giunta A, Stolfi C, Ballaro C, Papoutsaki M, Alema S, Monteleone G, Chimenti S, Karin M, Costanzo A (2008) The tumor suppressor activity of IKKalpha in stratified epithelia is exerted in part via the TGF-beta antiproliferative pathway. *Proc Natl Acad Sci USA* **105**(44): 17091–17096.
- Mulero MC, Ferres-Marco D, Islam A, Margalef P, Pecoraro M, Toll A, Drechsel N, Charneco C, Davis S, Bellora N, Gallardo F, Lopez-Arribillaga E, Asensio-Juan E, Rodilla V, Gonzalez J, Iglesias M, Shih V, Mar Alba M, Di Croce L, Hoffmann A, Miyamoto S, Villa-Freixa J, Lopez-Bigas N, Keyes WM, Dominguez M, Bigas A, Espinosa L (2013) Chromatin-bound IkappaBalpha regulates a subset of polycomb target genes in differentiation and cancer. *Cancer Cell* **24**(2): 151–166.
- Park E, Zhu F, Liu B, Xia X, Shen J, Bustos T, Fischer SM, Hu Y (2007) Reduction in IkappaB kinase alpha expression promotes the development of skin papillomas and carcinomas. *Cancer Res* **67**(19): 9158–9168.

- Rao P, Hayden MS, Long M, Scott ML, West AP, Zhang D, Oeckinghaus A, Lynch C, Hoffmann A, Baltimore D, Ghosh S (2010) I κ B α acts to inhibit and activate gene expression during the inflammatory response. *Nature* **466**(7310): 1115–1119.
- Rebholz B, Haase I, Eckelt B, Paxian S, Flaig MJ, Ghoreschi K, Nedospasov SA, Mailhammer R, Debey-Pascher S, Schultze JL, Weindl G, Forster I, Huss R, Stratis A, Ruzicka T, Rocken M, Pfeffer K, Schmid RM, Rupec RA (2007) Crosstalk between keratinocytes and adaptive immune cells in an I κ B α protein-mediated inflammatory disease of the skin. *Immunity* **27**(2): 296–307.
- Scheibel M, Klein B, Merkle H, Schulz M, Fritsch R, Greten FR, Arkan MC, Schneider G, Schmid RM (2010) I κ B α is an essential co-activator for LPS-induced IL-1 β transcription *in vivo*. *J Exp Med* **207**(12): 2621–2630.
- Seitz CS, Lin Q, Deng H, Khavari PA (1998) Alterations in NF- κ B function in transgenic epithelial tissue demonstrate a growth inhibitory role for NF- κ B. *Proc Natl Acad Sci USA* **95**(5): 2307–2312.
- Shih VF, Kearns JD, Basak S, Savinova OV, Ghosh G, Hoffmann A (2009) Kinetic control of negative feedback regulators of NF- κ B/RelA determines their pathogen- and cytokine-receptor signaling specificity. *Proc Natl Acad Sci USA* **106**(24): 9619–9624.
- Sil AK, Maeda S, Sano Y, Roop DR, Karin M (2004) I κ B kinase- α acts in the epidermis to control skeletal and craniofacial morphogenesis. *Nature* **428**(6983): 660–664.
- Toll A, Salgado R, Yebenes M, Martin-Ezquerria G, Gilaberte M, Baro T, Sole F, Alameda F, Espinet B, Pujol RM (2009) MYC gene numerical aberrations in actinic keratosis and cutaneous squamous cell carcinoma. *Br J Dermatol* **161**(5): 1112–1118.
- van Hogerlinden M, Rozell BL, Ahrlund-Richter L, Toftgard R (1999) Squamous cell carcinomas and increased apoptosis in skin with inhibited Rel/nuclear factor- κ B signaling. *Cancer Res* **59**(14): 3299–3303.
- van Hogerlinden M, Rozell BL, Toftgard R, Sundberg JP (2004) Characterization of the progressive skin disease and inflammatory cell infiltrate in mice with inhibited NF- κ B signaling. *J Invest Dermatol* **123**(1): 101–108.
- Wuerzberger-Davis SM, Chen Y, Yang DT, Kearns JD, Bates PW, Lynch C, Ladell NC, Yu M, Podd A, Zeng H, Huang TT, Wen R, Hoffmann A, Wang D, Miyamoto S (2011) Nuclear export of the NF- κ B inhibitor I κ B α is required for proper B cell and secondary lymphoid tissue formation. *Immunity* **34**(2): 188–200.
- Zhu F, Xia X, Liu B, Shen J, Hu Y, Person M (2007) IKK α shields 14-3-3 σ , a G(2)/M cell cycle checkpoint gene, from hypermethylation, preventing its silencing. *Mol Cell* **27**(2): 214–227.



This work is licensed under the Creative Commons Attribution-NonCommercial-Share Alike 3.0 Unported License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-sa/3.0/>