Shostak and Chariot *Breast Cancer Research* 2011, **13**:214
http://breast-cancer-research.com/content/13/4/214

Breast Cancer RESEARCH

REVIEW

NF-κB, stem cells and breast cancer: the links get stronger

Kateryna Shostak and Alain Chariot*

Abstract

Self-renewing breast cancer stem cells are key actors in perpetuating tumour existence and in treatment resistance and relapse. The molecular pathways required for their maintenance are starting to be elucidated. Among them is the transcription factor NF-κB, which is known to play critical roles in cell survival, inflammation and immunity. Recent studies indicate that mammary epithelial NF-κB regulates the self-renewal of breast cancer stem cells in a model of Her2-dependent tumourigenesis. We will describe here the NF-κB-activating pathways that are involved in this process and in which progenitor cells this transcription factor is actually activated.

Breast cancer is a heterogeneous disease, yet it remains possible to highlight common molecular signatures from distinct tumour subtypes. A frequent feature found in most breast cancer tumours is the constitutive activation of NF-κB, a family of transcription factors that play critical roles in cell survival, proliferation, inflammation and immunity [1]. Deregulated NF-κB activation results in the persistent nuclear localization of proteins such as p50, p52, p65, cRel and RelB, which leads to the disruption of the balance between cell proliferation and death through the upregulation of anti-apoptotic proteins [2].

The main NF-κB-activating pathways

Two major NF-κB-activating pathways have been characterized, referred to as the classical or canonical and the alternative or non-canonical pathways. Both rely on the signal-induced phosphorylation and degradation of an inhibitory molecule and the subsequent release and nuclear shuttling of NF-κB proteins. Yet, both pathways differ by the signals that trigger them as well as by the identity of the activated kinases, the inhibitory molecule and the NF-κB proteins. The classical pathway is typically triggered by pro-inflammatory cytokines such as TNFα or IL-1β and ultimately leads to the degradation of the inhibitory molecule IκBα by the NF-κB essential modulator (NEMO)/IκB kinase (IKK)γ-containing IKK complex through a TAK1-dependent pathway [1] (Figure 1). The p50/p65 heterodimer will then move into the nucleus to induce the expression of genes involved in cell proliferation and survival, inflammation and innate immunity. The alternative pathway triggers the partial degradation of the inhibitory molecule p100 into p52 through a NF-κB-inducing kinase (NIK)-dependent pathway (Figure 1). This cascade relies on an IKKα heterodimer but not on NEMO/IKKγ and ultimately leads to the nuclear shuttling of p52/RelB dimers. This signalling pathway plays a critical role in adaptive immunity [1].

The classical NF-κB-activating pathway in breast cancer

Based on the key role of NF-κB in mammary epithelial proliferation, architecture and branching during early post-natal development [3,4], it was not surprising to see that the constitutive NF-κB activation found in several breast tumour cell lines has profound consequences in the initiation and progression of breast cancer [5]. NF-κB is mostly activated in oestrogen receptor-negative (ER-negative) and ErbB2-positive tumours [6,7]. Importantly, a NEMO-binding domain (NBD) peptide, which acts as a selective inhibitor of the IKK complex, blocked heregulin-mediated NF-κB activation and induced apoptosis preferentially in proliferating cells, showing that the classical pathway largely contributes to tumour development [6]. Those initial reports were followed by studies that more specifically addressed the role of NF-κB in breast tumour development *in vivo*. A genetic approach in which the classical NF-κB-activating pathway is inhibited in defined windows during polyoma middle T oncogene (PyVT) tumourigenesis showed that interfering with this pathway increases tumour latency and decreases tumour burden [8]. These findings are in agreement with data showing the requirement of NF-κB for the induction and

*Correspondence: Alain.chariot@ulg.ac.be
Interdisciplinary Cluster for Applied Genoproteomics (GIGA-Research), Unit of Medical Chemistry and GIGA-Signal Transduction, University of Liege, CHU, Sart-Tilman, 4000 Liège, Belgium

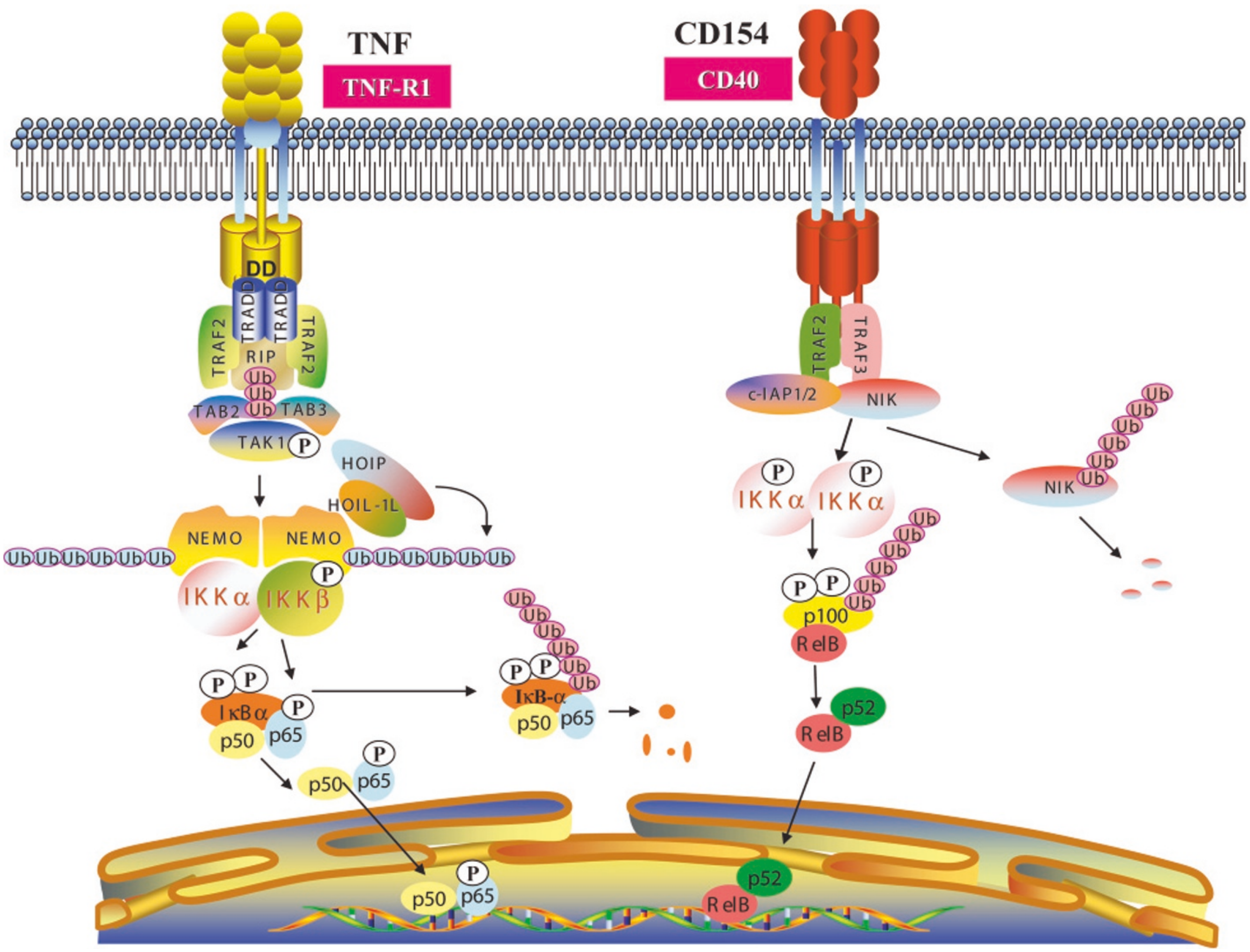

Figure 1. The main NF-κB-activating pathways. On the left is the TNFα-dependent signalling pathway. The binding of TNFα to the TNF receptor TNFR1 triggers the sequential recruitment of the adaptors TRADD (TNFR1-associated death domain protein), RIP and TRAF2 (TNF receptor-associated factor 2) to the membrane. Then, TRAF2 mediates the recruitment of the IκB kinase (IKK) complex, composed of IKKα, IKKβ and NEMO (NF-kappa-B essential modulator), to the TNFR1 signalling complex. The scaffold proteins TAB2 and TAB3 subsequently bind to Lys63-polyubiquitylated substrates, such as receptor-interacting protein (RIP)1, resulting in TAK1 and then IKKβ activation. NEMO actually exerts its essential role in NF-κB activation by integrating upstream IKK-activating signals. Importantly, the linear ubiquitin (Ub) chain assembly complex (LUBAC), composed of two proteins, namely HOIL-1L (heme-oxidized IRP2 ubiquitin ligase-1) and HOIP (HOIL-1L interacting protein), binds NEMO in a TNFα-dependent manner, and generates and conjugates linear chains of ubiquitin on the scaffold protein of the IKK complex [42]. The ubiquitin-binding motif of NEMO, referred to as the UBAN motif, is required to sense linear chains of ubiquitin. Activation of IKKβ leads to IκBα phosphorylation on specific residues, polyubiquitylation through binding of ubiquitin proteins and its degradation through the proteasome pathway. Then, the heretodimer p50-p65 binds to specific κB sites and activates a variety of NF-κB target genes coding for pro-inflammatory cytokines (IL-6) and chemokines. On the right is the alternative NF-κB-activating pathway. Binding of CD154 triggers the classical NEMO-dependent pathway (not illustrated) and the NEMO-independent cascade. This pathway relies on the recruitment of the heterodimer TRAF2-TRAF3 to the CD40 receptor. TRAF3 is required to connect the E3 ligases c-IAP1/2 (cellular inhibitor of apoptosis 1/2) to the kinase NIK (NF-κB-inducing kinase). NIK is activated by phosphorylation and is also subjected to a c-IAP1/2-dependent degradative polyubiquitination. IKKα homodimers are activated by NIK and phosphorylate the inhibitory molecule p100, the partial processing of which generates the NF-κB protein p52. This latter transcription factor moves into the nucleus as a heterodimer with RelB to regulate the expression of genes involved in lymphoid organogenesis or coding for chemokines (BLC (B lymphocyte chemokine)) or cytokines (BAFF (B-cell activating factor)).

maintenance of the epithelial-mesenchymal transition (EMT), a process that critically controls breast cancer progression [9,10]. Indeed, the MCF10A immortalized cell line, which is derived from normal mammary epithelial cells, undergoes EMT when overexpressing the NF-κB protein p65. This latter protein suppresses the expression of epithelial markers such as E-cadherin and desmoplakin but also induces the expression of mesenchymal markers such as vimentin. This process may occur through the NF-κB-dependent expression of ZEB-1/ZFHX1A and ZEB-2/ZFHX1B/Smad-interacting protein (SIP1), two transcriptional regulators known to repress E-cadherin expression and to promote EMT [10]. These data strongly suggest that NF-κB regulates breast tumour progression independently of its effects on mammary development.

The alternative NF-κB-activating pathway in breast cancer

The classical NF-κB-activating pathway is not the only one that contributes to breast cancer development. Indeed, early studies also demonstrated enhanced expression of the NF-κB protein p52 in breast cancer samples [11,12]. Moreover, increased p52/RelB activity was also observed in mouse mammary tumours induced by 7,12-dimethylbenz(*a*)anthracene (DMBA) [13]. The definitive proof that the alternative NF-κB-activating pathway is involved in breast cancer development came from the phenotype of a mouse transgenic model in which p100/p52 is specifically overexpressed in the mammary epithelium by using the β-lactoglobulin milk protein promoter [14]. This mouse model not only showed a delay in mammary development but also a transient reduction in ductal branching during pregnancy. Matrix metalloproteinase (Mmp)-2, Mmp-9 and cyclooxygenase (Cox)-2 turned out to be overexpressed in these transgenic mice. Constitutive p100 overexpression causes an aberrant phenotype, as shown by the thickening of primary ducts, loss of epithelial cell organization and small areas of hyperplastic growth. Finally, an increase in p100/p52 expression was also observed in PyVT mice when tumour development is observed [14]. Importantly, no change of nuclear p65 was detected in this mouse model, suggesting that the phenotype observed was exclusively the result of a deregulated alternative NF-κB-activating pathway. The expression of the NF-κB protein RelB, which is known to play a critical role in this signalling cascade, is increased in ERα-negative breast cancer cells [15]. ERα actually represses NF-κB and AP-1 activities and consequently RelB expression. Interestingly, RelB is required for the maintenance of the mesenchymal phenotype of ERα-negative Hs578T breast cancer cells, at least in part through the transcriptional induction of BCL2 [15].

Other NF-κB-activating pathways in breast cancer

NF-κB is not exclusively activated through the TNF family of receptors. Indeed, the binding of epidermal growth factor (EGF) to its receptor (EGFR) also ultimately activates NF-κB and most likely contributes to the enhanced activity of this transcription factor in ER-negative breast cancer cells [16]. The exact mechanism by which EGF activates NF-κB in breast cancer cells remains unclear but may be similar to that described in lung cancer cells. EGF appears to trigger IκBα phosphorylation on tyrosine 42 through an IKK-independent pathway [17]. Of note, the inhibitory molecule ABIN-1 also negatively regulates EGF-mediated NF-κB activation, a pathway that requires its carboxy-terminal ubiquitin-binding domain [18].

The IKK complex is not the only one whose activation is often constitutive in breast cancer. Indeed, the so called IKK-related kinase IKKε is also overexpressed in some cases of breast adenocarcinomas as the result of the 1q32 amplicon [19]. This gene amplification is not the only mechanism by which this kinase is aberrantly expressed, as more than 45% of IKKε-overexpressing breast carcinomas do not harbour the 1q32 amplicon. IKKε expression can be induced by casein kinase 2 (CK2) in breast cancer cells [20] and other pathways still to be characterized may also contribute to this phenomenon. Interestingly, this kinase is known to promote type I interferon gene induction through IRF3 phosphorylation, acts downstream of Akt and activates NF-κB by facilitating the nuclear localization of c-REL [19,20]. Importantly, all IKKε substrates identified in breast cancer samples so far act as signalling molecules in NF-κB-dependent cascades and mediate the oncogenic potential of this IKK-related kinase. CYLD, a NF-κB inhibitor acting as a tumour suppressor, is phosphorylated by IKKε, a modification that decreases its deubiquitine ligase activity and consequently its inhibitory potential [21]. Therefore, an extensive understanding of the role of NF-κB in breast cancer development and progression should not be limited to the characterization of both classical and alternative pathways.

NF-κB and cancer stem cells

Self-renewing breast cancer stem cells are the subject of intensive research as key actors responsible for perpetuating tumour existence and for treatment resistance and relapse. These cells can be isolated by virtue of their expression of the cell surface markers epithelial-specific antigen (ESA) and CD44 and the absence of expression of CD24. The expression of aldehyde dehydrogenase has also been used to enrich for tumour-initiating cells and revealed that distinct breast cancers may contain cancer stem cells that harbour different cell surface markers [22,23]. Importantly, stem cell properties can also be gained by transformed cells undergoing EMT [24].

At the molecular level, Wnt, Notch and Hedgehog developmental pathways control the self-renewal of normal stem cells and also appear to be deregulated in many human breast cancers [25]. Moreover, the membrane bound receptor tyrosine kinase Her2, which is overexpressed in 30% of breast cancers, also critically controls the cancer stem-cell population [26]. As Her2 activates NF-κB through the canonical pathway [27], the hypothesis that this latter family of proteins may be involved in the biology of breast cancer stem cells made sense. This issue was recently addressed in a mouse model of Her2 breast tumourigenesis in which NF-κB was temporally suppressed in the mammary gland [28]. This approach was elegant as NF-κB activation through the canonical pathway was only suppressed in mammary epithelial cells but not in inflammatory cells, blood vessels or adipocytes, where this transcription factor most likely contributes to tumour development. Moreover, NF-κB suppression was inducible to circumvent the requirement of this transcription factor in normal ductal development. The authors first noticed that NF-κB is required for cell proliferation and colony formation of Her2-derived murine mammary tumour cell lines [28]. They subsequently observed that NF-κB governs the rate of initiation of Her2 tumours through multiple pathways ranging from reactive oxygen species production to cellular proliferation, invasion, inflammation and vasculogenesis. Mammary epithelial NF-κB contributed to the recruitment of tumour-associated macrophages. Interestingly, the proportion of CD44-positive cells dramatically decreased in Her2-dependent tumours where NF-κB was suppressed, thus indicating that this transcription factor maintains progenitor cell expansion [28]. This result was further supported by the reduced formation of non-adherent mammospheres with cell lines derived from Her2-dependent tumours in which NF-κB was inhibited. This phenomenon was potentially due to the reduced expression of key embryonic stem cell regulators such as Sox2 and Nanog [28]. As this study was based on the expression of an IκBα super repressor in which both serines 32 and 36 were mutated to alanines, the resulting phenotype was caused by a defective NF-κB and IKKβ-dependent activating pathway. Yet, this signalling cascade is not the only one that contributes to breast cancer stem cell expansion. Indeed, IKKα$^{AA/AA}$ knockin mice in which IKKα activation is disrupted by replacement of activation loop serines by alanines showed delayed tumour development when crossed with the Her2 murine breast cancer model [29]. Breast cancer cells from these mice generated primary but not secondary mammospheres, suggesting that IKKα is also required for the self-renewal of tumour-initiating cells from the Her2 breast cancer model.

IKKα appears to act as a central NF-κB-activating protein in the self-renewal of breast cancer stem cells, as evidenced by data obtained from additional mouse models of breast cancer. Indeed, deletion of IKKα in mammary-gland epithelial cells affects the onset of progestin-driven breast cancer [30]. This kinase is actually activated through the Receptor activator of nuclear factor kappa-B ligand (RANKL)/RANK pathway when progesterone or synthetic derivatives (progestins) such as medroxyprogesterone acetate (MPA) are given in combination with the DNA-damaging agent DMBA to mice. As a result, cell proliferation occurs through *cyclin D1* gene induction [30]. Interestingly, treatment with MPA alone led to a significant expansion of luminal progenitor cells through a massive induction of RANKL, a phenomenon that was impaired in females defective for RANK. Mouse mammary tumour virus (MMTV)-RANK transgenic mice showed an enhanced susceptibility to mammary tumours following a MPA/DMBA treatment whereas RANK invalidation in the mammary gland resulted in a delayed onset and decreased incidence of progestin-driven breast cancers [30,31]. Importantly, breast cancer cells from MPA and DMBA-treated RANK-defective mice formed primary but not secondary mammospheres, strongly suggesting that a loss of RANK expression markedly impairs the self-renewal capacity of cancer stem cells [30].

NF-κB appears to be activated during differentiation of the mammary colony-forming cells in which luminal progenitor cells can be found [32] (Figure 2). On the other hand, the mammary stem-like basally located cells, also known as mammary repopulating units, are devoid of NF-κB activity [32,33].

As a transcription factor required for the production of chemokines and cytokines, NF-κB has been defined as an essential actor in the link between inflammation and oncogenesis initiation and progression [34]. Indeed, inflammatory molecules such as IL6 provide growth signals that promote malignant cell proliferation. Interestingly, a transient activation of the kinase oncoprotein Src in MCF10A cells results in phenotypic transformation that includes the formation of multiple foci, the ability to form colonies in soft agar and tumours in xenografts as well as mammosphere formation [35]. This epigenetic switch, defined when a stable cell type changes into another stable cell type without any modification in DNA sequences, involves a rapid inflammation response that requires NF-κB [35]. More specifically, NF-κB activation triggers *Lin28B* expression, which in turn decreases Let-7 microRNA levels. As Let-7 microRNA directly targets IL6 mRNAs by binding their 3' untranslated region, the IL6-dependent signalling pathways are strongly induced through the Src- and NF-κB-dependent cascade [35]. This newly defined pathway appears to play a key role in the self-renewal capacity of breast cancer stem cells. Therefore, this study not only defined Src as an oncogenic kinase that promotes the expansion of breast cancer stem cells but also demonstrated how critical NF-κB is in this process.

Unclear issues

Despite significant progress in the elucidation of the roles played by NF-κB in breast cancer stem cell expansion, some issues remain to be experimentally addressed. The gene candidates known to regulate embryonic stem cells

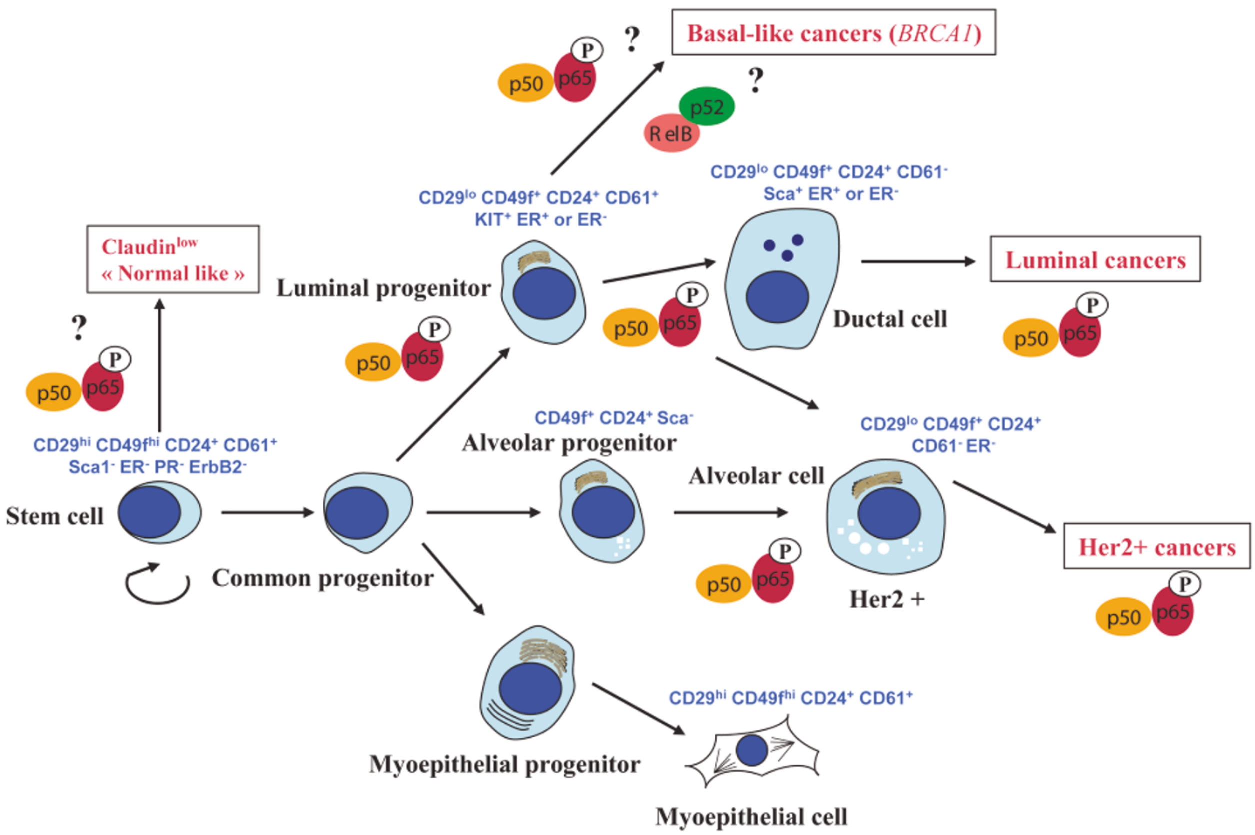

Figure 2. Hypothetical model of the differentiation hierarchy within the mammary epithelium and potential relationships with breast cancer subtypes. NF-κB activation through the canonical or the non-canonical pathway is depicted as p50/p65 or p52/RelB heterodimers, respectively. Cell surface markers used for the isolation of human epithelial cell subsets are shown in blue. Adapted from [32,33]. ER, oestrogen receptor; PR, progesterone receptor.

and to be specifically induced through IKKα activation in the mammary gland remain to be identified. Are Sox2 and Nanog induced by both IKKα and IKKβ? Cyclin D1, whose expression is strongly impaired in the IKK$\alpha^{AA/AA}$ knockin mouse [4], may be one promising candidate as its kinase activity appears to be crucial for the self-renewal of mammary stem and progenitor cells [36]. It also remains to be seen whether the alternative pathway is truly involved in breast cancer stem cell expansion. Based on the fact that the Her-2-dependent NF-κB activation pathway surprisingly relies on IKKα but not on IKKβ [27], the phenotype observed in the IKK$\alpha^{AA/AA}$ knockin mouse crossed with the Her2 breast cancer model may actually be the result of a defective classical rather than alternative NF-κB activation cascade. In agreement with this hypothesis, the IKK$\alpha^{AA/AA}$ mutation in mammary epithelial cells results in decreased nuclear levels of p50 and p65, the proteins acting in the classical pathway.

Most of the data showing a link between NF-κB and breast cancer stem cells were obtained using the Her-2 tumour model, which classifies with human luminal-type breast cancers. The canonical NF-κB pathway is active in normal luminal progenitor cells and is consequently required for the formation of mammary epithelial tumours [32]. On the other hand, NF-κB appears to be dispensable for tumour development and progression in the MMTV-Wnt1 model, which classifies with human basal-like breast cancers [29]. This is most likely due to the absence of nuclear p65 in these lesions as well as in the mammary stem-like basally located cells, also referred to as mammary repopulating units [32]. The molecular mechanisms underlying the specific NF-κB activation in luminal progenitor cells remains to be elucidated. The following years will provide further insights into the biology of both multipotent stem cells and lineage-committed progenitor cells and will tell us why and how NF-κB regulates their functions.

The IKK-related kinase IKKε appears to act as an oncogenic protein through NF-κB, yet it is totally unclear whether and how this kinase promotes breast cancer stem cell expansion. The generation of a new mouse model specifically harbouring the 1q32 amplicon may be useful to address this issue. Recent studies indicated that the IKK complex targets multiple substrates and therefore has additional NF-κB-independent functions in cancer [37]. Whether and how these unexpected roles contribute to breast cancer stem cell maintenance also remains an open question.

Important studies dedicated to the identification of the cell of origin of solid tumours, including breast cancers, have been published [38]. The cell of origin may correspond to the normal tissue stem cell, which can take advantage of its intrinsic self-renewal capacity. A restricted progenitor can, for other malignancies, act as the initiating cell, as reported, for example, for *BRCA1*-associated breast cancers [39,40]. Breast cancers in the *BRCA1* mutation carriers have a distinct basal-like phenotype, yet these tumours originated from luminal epithelial progenitors instead of from basal stem cells [39,40]. It remains to be seen whether and how NF-κB would be required for the conversion of a luminal progenitor cell to a basal gene expression profile in tumours originated in *BRCA1* mutation carriers. Nevertheless, identification of the cell origin is a key step to develop preventive therapeutic approaches in order to suppress or reverse the initial phase of the disease. In this context, the identification of the RANKL/RANK/IKKα/NF-κB axis as a key pathway that provides paracrine signals to stem cells means that it is conceivable to prevent some cases of breast cancers by driving stem cells into a dormant state. This strategy could be achieved by interfering with the RANK pathway with some specific inhibitors, some of which are already in clinical trials for bone metastasis [38].

An extensive knowledge of the biology of breast cancer stem cells is also crucial in order to better understand why some patients develop resistance to therapy such as Her2-targeting agents. Resistance to these drugs is the result of various somatic mutations that ultimately trigger aberrant phosphoinositide 3-kinase (PI3K)/Akt activation [41]. Although Akt appears to be dispensable for Her2-mediated NF-κB activation in breast cancer cells [27], this transcription factor is nevertheless a key actor in chemoresistance. Future studies should also tell us whether and how NF-κB inhibition in breast cancer stem cells modulates their resistance to therapy.

Abbreviations

DMBA, 7,12-dimethylbenz(*a*)anthracene; EGF, epidermal growth factor; EMT, epithelial-mesenchymal transition; ER, oestrogen receptor; IKK, IκB kinase; IL, interleukin; MMTV, mouse mammary tumour virus; MPA, medroxyprogesterone acetate ; NEMO, NF-kappa-B essential modulator; NF, nuclear factor; NIK, NF-κB-inducing kinase; PyVT, polyoma middle T oncogene; RANK, Receptor activator of nuclear factor kappa-B; RANKL, Receptor activator of nuclear factor kappa-B ligand; RIP, receptor-interacting protein; TNF, tumour necrosis factor.

Competing interests

The authors declare that they have no competing interests.

Published: 26 July 2011

References

1. Hayden MS, Ghosh S: Shared principles in NF-kappa B signaling. *Cell* 2008, **132**:344-362.
2. Karin M, Lin A: NF-kappa B at the crossroads of life and death. *Nat Immunol* 2002, **3**:221-227.
3. Brantley DM, Chen CL, Muraoka RS, Bushdid PB, Bradberry JL, Kittrell F, Medina D, Matrisian LA, Kerr LD, Yull FE: Nuclear factor-kB (NF-kB) regulates proliferation and branching in mouse mammary epithelium. *Mol Biol Cell* 2001, **12**:1445-1455.
4. Cao YX, Bonizzi G, Seagroves TN, Greten FR, Johnson R, Schmidt EV, Karin M: IKK alpha provides an essential link between RANK signaling and cyclin D1 expression during mammary gland development. *Cell* 2001, **107**:763-775.
5. Sovak MA, Bellas RE, Kim DW, Zanieski GJ, Rogers AE, Traish AM, Sonenshein GE: Aberrant nuclear factor-kappa B/Rel expression and the pathogenesis of breast cancer. *J Clin Invest* 1997, **100**:2952-2960.
6. Biswas DK, Shi Q, Baily S, Strickland I, Ghosh S, Pardee AB, Iglehart JD: NF-kB activation in human breast cancer specimens and its role in cell proliferation and apoptosis. *Proc Natl Acad Sci U S A* 2004, **101**:10137-10142.
7. Nakshatri H, BhatNakshatri P, Martin DA, Goulet RJ, Sledge GW: Constitutive activation of NF-kappa B during progression of breast cancer to hormone-independent growth. *Mol Cell Biol* 1997, **17**:3629-3639.
8. Connelly L, Barham W, Onishko HM, Sherrill T, Chodosh LA, Blackwell TS, Yull FE: Inhibition of NF-kappa B activity in mammary epithelium increases tumor latency and decreases tumor burden. *Oncogene* 2011, **30**:1402-1412.
9. Huber MA, Azoitei N, Baumann B, Grunert S, Sommer A, Pehamberger H, Kraut N, Beug H, Wirth T: NF-kappa B is essential for epithelial-mesenchymal transition and metastasis in a model of breast cancer progression. *J Clin Invest* 2004, **114**:569-581.
10. Chua HL, Bhat-Nakshatri P, Clare SE, Morimiya A, Badve S, Nakshatri H: NF-kappa B represses E-cadherin expression and enhances epithelial to mesenchymal transition of mammary epithelial cells: potential involvement of ZEB-1 and ZEB-2. *Oncogene* 2007, **26**:711-724.
11. Dejardin E, Bonizzi G, Bellahcene A, Castronovo V, Merville MP, Bours V: Highly-expressed P100/P52 (Nfkb2) sequesters other Nf-Kappa-B-related proteins in the cytoplasm of human breast-cancer cells. *Oncogene* 1995, **11**:1835-1841.
12. Cogswell PC, Guttridge DC, Funkhouser WK, Baldwin AS: Selective activation of NF-kappa B subunits in human breast cancer: potential roles for NF-kappa B2/p52 and for Bcl-3. *Oncogene* 2000, **19**:1123-1131.
13. Demicco EG, Kavanagh KT, Romieu-Mourez R, Wang XB, Shin SR, Landesman-Bollag E, Seldin DC, Sonenshein GE: RelB/p52 NF-kappa B complexes rescue an early delay in mammary gland development in transgenic mice with targeted superrepressor I kappa B-alpha expression and promote carcinogenesis of the mammary gland. *Mol Cell Biol* 2005, **25**:10136-10147.
14. Connelly L, Robinson-Benion C, Chont M, Saint-Jean L, Li HJ, Polosukhin VV, Blackwell TS, Yull FE: A transgenic model reveals important roles for the NF-kappa B alternative pathway (p100/p52) in mammary development and links to tumorigenesis. *J Biol Chem* 2007, **282**:10028-10035.
15. Wang XB, Belguise K, Kersual N, Kirsch KH, Mineva ND, Galtier F, Chalbos D, Sonenshein GE: Oestrogen signalling inhibits invasive phenotype by repressing RelB and its target BCL2. *Nat Cell Biol* 2007, **9**:470-U197.
16. Biswas DK, Cruz AP, Gansberger E, Pardee AB: Epidermal growth factor-induced nuclear factor kappa B activation: A major pathway of cell-cycle progression in estrogen-receptor negative breast cancer cells. *Proc Natl Acad Sci U S A* 2000, **97**:8542-8547.
17. Sethi G, Ahn KS, Chaturvedi MM, Aggarwal BB: Epidermal growth factor (EGF) activates nuclear factor-kappa B through I kappa B alpha kinase-independent but EGF receptor-kinase dependent tyrosine 42 phosphorylation of I kappa B alpha. *Oncogene* 2007, **26**:7324-7332.
18. Huang L, Verstrepen L, Heyninck K, Wullaert A, Revets H, De Baetselier P, Beyaert R: ABINs inhibit EGF receptor-mediated NF-kappa B activation and growth of EGF receptor-overexpressing tumour cells. *Oncogene* 2008, **27**:6131-6140.
19. Boehm JS, Zhao JJ, Yao J, Kim SY, Firestein R, Dunn IF, Sjostrom SK, Garraway LA, Weremowicz S, Richardson AL, Greulich H, Stewart CJ, Mulvey LA, Shen

RR, Ambrogio L, Hirozane-Kishikawa T, Hill DE, Vidal M, Meyerson M, Grenier JK, Hinkle G, Root DE, Roberts TM, Lander ES, Polyak K, Hahn WC: Integrative genomic approaches identify IKBKE as a breast cancer oncogene. *Cell* 2007, **129**:1065-1079.
20. Eddy SF, Guo SQ, Demicco EG, Romieu-Mourez R, Landesman-Borag E, Seldin DC, Sonenshein GE: Inducible I kappa B kinase/I kappa B kinase epsilon expression is induced by CK2 and promotes aberrant nuclear factor-kappa B activation in breast cancer cells. *Cancer Res* 2005, **65**:11375-11383.
21. Hutti JE, Shen RR, Abbott DW, Zhou AY, Sprott KM, Asara JM, Hahn WC, Cantley LC: Phosphorylation of the tumor suppressor CYLD by the breast cancer oncogene IKK epsilon promotes cell transformation. *Mol Cell* 2009, **34**:461-472.
22. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, Jacquemier J, Viens P, Kleer CG, Liu S, Schott A, Hayes D, Birnbaum D, Wicha MS, Dontu G: ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell* 2007, **1**:555-567.
23. Charafe-Jauffret E, Ginestier C, Iovino F, Wicinski J, Cervera N, Finetti P, Hur MH, Diebel ME, Monville F, Dutcher J, Brown M, Viens P, Xerri L, Bertucci F, Stassi G, Dontu G, Birnbaum D, Wicha MS: Breast cancer cell lines contain functional cancer stem cells with metastatic capacity and a distinct molecular signature. *Cancer Res* 2009, **69**:1302-1313.
24. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Brisken C, Yang J, Weinberg RA: The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008, **133**:704-715.
25. Liu SL, Dontu G, Wicha MS: Mammary stem cells, self-renewal pathways, and carcinogenesis. *Breast Cancer Res* 2005, **7**:86-95.
26. Korkaya H, Paulson A, Iovino F, Wicha MS: HER2 regulates the mammary stem/progenitor cell population driving tumorigenesis and invasion. *Oncogene* 2008, **27**:6120-6130.
27. Merkhofer EC, Cogswell P, Baldwin AS: Her2 activates NF-kappa B and induces invasion through the canonical pathway involving IKK alpha. *Oncogene* 2010, **29**:1238-1248.
28. Liu M, Sakamaki T, Casimiro MC, Willmarth NE, Quong AA, Ju X, Ojeifo J, Jiao X, Yeow WS, Katiyar S, Shirley LA, Joyce D, Lisanti MP, Albanese C, Pestell RG: The canonical NF-kappa B pathway governs mammary tumorigenesis in transgenic mice and tumor stem cell expansion. *Cancer Res* 2010, **70**:10464-10473.
29. Cao YX, Luo JL, Karin M: I kappa B kinase a kinase activity is required for self-renewal of ErbB2/Her2-transformed mammary tumor-initiating cells. *Proc Natl Acad Sci U S A* 2007, **104**:15852-15857.
30. Schramek D, Leibbrandt A, Sigl V, Kenner L, Pospisilik JA, Lee HJ, Hanada R, Joshi PA, Aliprantis A, Glimcher L, Pasparakis M, Khokha R, Ormandy CJ, Widschwendter M, Schett G, Penninger JM: Osteoclast differentiation factor RANKL controls development of progestin-driven mammary cancer. *Nature* 2010, **468**:98-102.
31. Gonzalez-Suarez E, Jacob AP, Jones J, Miller R, Roudier-Meyer MP, Erwert R, Pinkas J, Branstetter D, Dougall WC: RANK ligand mediates progestin-induced mammary epithelial proliferation and carcinogenesis. *Nature* 2010, **468**:103-107.
32. Pratt MAC, Tibbo E, Robertson SJ, Jansson D, Hurst K, Perez-Iratxeta C, Lau R, Niu MY: The canonical NF-kappa B pathway is required for formation of luminal mammary neoplasias and is activated in the mammary progenitor population. *Oncogene* 2009, **28**:2710-2722.
33. Visvader JE: Keeping abreast of the mammary epithelial hierarchy and breast tumorigenesis. *Gene Dev* 2009, **23**:2563-2577.
34. Naugler WE, Karin M: NF-kappa B and cancer - identifying targets and mechanisms. *Curr Opin Genet Dev* 2008, **18**:19-26.
35. Iliopoulos D, Hirsch HA, Struhl K: An epigenetic switch involving NF-kappa B, Lin28, Let-7 microRNA, and IL6 links inflammation to cell transformation. *Cell* 2009, **139**:693-706.
36. Jeselsohn R, Brown NE, Arendt L, Klebba I, Hu MG, Kuperwasser C, Hinds PW: Cyclin D1 kinase activity is required for the self-renewal of mammary stem and progenitor cells that are targets of MMTV-ErbB2 tumorigenesis. *Cancer Cell* 2010, **17**:65-76.
37. Chariot A: The NF-kappa B-independent functions of IKK subunits in immunity and cancer. *Trends Cell Biol* 2009, **19**:404-413.
38. Visvader JE: Cells of origin in cancer. *Nature* 2011, **469**:314-322.
39. Lim E, Vaillant F, Wu D, Forrest NC, Pal B, Hart AH, Asselin-Labat ML, Gyorki DE, Ward T, Partanen A, Feleppa F, Huschtscha LI, Thorne HJ; kConFab, Fox SB, Yan M, French JD, Brown MA, Smyth GK, Visvader JE, Lindeman GJ: Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. *Nat Med* 2009, **15**:907-913.
40. Molyneux G, Geyer FC, Magnay FA, McCarthy A, Kendrick H, Natrajan R, Mackay A, Grigoriadis A, Tutt A, Ashworth A, Reis-Filho JS, Smalley MJ: BRCA1 basal-like breast cancers originate from luminal epithelial progenitors and not from basal stem cells. *Cell Stem Cell* 2010, **7**:403-417.
41. Nagata Y, Lan KH, Zhou X, Tan M, Esteva FJ, Sahin AA, Klos KS, Li P, Monia BP, Nguyen NT, Hortobagyi GN, Hung MC, Yu D: PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell* 2004, **6**:117-127.
42. Iwai K, Tokunaga F: Linear polyubiquitination: a new regulator of NF-kappa B activation. *EMBO Rep* 2009, **10**:706-713.

doi:10.1186/bcr2886
Cite this article as: Shostak K, Chariot A: NF-κB, stem cells and breast cancer: the links get stronger. *Breast Cancer Research* 2011, **13**:214.