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NFκB and MAPK signalling pathways mediate TNFα-induced Early Growth Response gene transcription leading to aromatase expression

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ABSTRACT

The Early Growth Response genes *EGR2* and *EGR3* play an important role in mediating TNFα induced aromatase expression via the adipose specific promoter PI.4. The upstream signalling pathway stimulated by TNFα to initiate this is unknown. The aim of this present study was to determine the signalling pathways activated by TNFα which result in *EGR2* and *EGR3* transcription, and ultimately activation of PI.4. The NFκB inhibitor BAY-11-7082 dose-dependently inhibited transcription of *EGR2* and *EGR3* mRNA as well as total and PI.4-specific *CYP19A1* mRNA. The MAPK pathway inhibitor U0126, inhibitor of MEK1/2 had the same effect, however inhibition of c-Jun and JNK1,2,3 with SP600125 did not lead to down-regulation. We provide evidence for the first time that *EGR2* and *EGR3* are regulated by NFκB and MAPK signalling pathways downstream of TNFα stimulation in breast adipose fibroblasts, and that this in turn is upstream of *CYP19A1* transcription via PI.4.

1. Introduction

Approximately 70% of post-menopausal breast cancers are diagnosed as Estrogen Receptor-α positive (ER+), and such tumours are responsive to estrogen for continued growth and proliferative advantage. Adjuvant selective estrogen receptor modulators (SERMs), such as Tamoxifen, are commonly used to block the action of estrogen in breast cancer [1]. ER+ tumours primarily source estrogen from the increased local production in surrounding breast adipose fibroblasts (BAFs) [2].

Aromatase is the key enzyme responsible for the conversion of androgens to estrogens. Its expression is regulated at the transcriptional level by its encoding gene *CYP19A1*. Upstream of the coding region, *CYP19A1* contains a number of tissue-specific promoters which are activated by distinct stimulatory factors in different tissues to convey a specific expression pattern [3,4]. In normal breast adipose and increasingly so in BAFs adjacent to an ER+ tumour, *CYP19A1* is transcribed via its distal adipose-specific promoter I.4 (PI.4), which is located approximately 73 kb upstream of the common splice site [5].

The activity of PI.4 is stimulated through the combined actions of glucocorticoids, such as dexamethasone, and cytokines [6,7], one of which is the pro-inflammatory cytokine tumour necrosis factor α (TNFα) [8]. The specific mechanisms by which TNFα is able to stimulate PI.4 activity remains to be resolved, however we recently demonstrated that the Early Growth Response (EGR) genes form an integral part of the pathway. EGR transcription factors, in particular EGR2 and EGR3, are upregulated upon TNFα stimulation in BAFs, and they in turn increase activity of PI.4 though not through direct binding to the promoter. Additionally, siRNA mediated knockdown of the EGR family members in BAFs significantly decreases expression of *CYP19A1* via PI.4 [9].

Whilst the first characterised family member EGR1 has been extensively studied, little is known about the regulation and biological function of other family members. Structurally similar, each EGR family member contains three zinc-finger DNA binding motifs which recognise the same CG-rich consensus sequence. Transcriptional regulation of EGR2 and EGR3 in the breast is poorly characterised, however EGR3 is thought to be an estrogen responsive gene in the ER+ breast cancer cell line MCF7 [10]. Understanding the molecular mechanisms by which EGRs are activated in the breast is important as it will help develop a clearer picture of how aromatase is upregulated, ultimately leading to the development of novel breast cancer treatments.

TNFα functions via a number of signalling pathways downstream of its main receptor, TNFR1. The best characterised of these are the Nuclear Factor κ-B (NFκB) and Mitogen Activated Protein Kinase (MAPK) signalling pathways. NFκB is hyperactivated in cases of breast cancer, promoting growth through hyper-activation of anti-apoptotic genes [11]. Compared to normal breast, breast cancers contain higher levels of activated Bcl-3, p65, p50 and

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p52, resulting in increased cyclin D1 levels and consequently cell cycle progression [12,13]. Experiments in mouse models of breast cancer have shown that application of the selective NFκB pathway inhibitor BAY-11-7082 can block TNFα-stimulated cell proliferation and cause tumour regression [14]. Components of the MAPK signalling cascade have also been targeted to slow the progression of tumour growth. p38 MAPK is highly overexpressed in both human and mouse metastatic breast cancer cell lines, and correlated with metastatic disease in clinical samples. shRNA mediated knockdown of p38 MAPK in breast cancer cell lines inhibits their proliferation, migration, invasion and colony forming capacity [15]. Inhibition of p38 MAPK signalling with the drug RWJ67657 strongly decreases tumour growth in a mouse xenograft model [16], indicating a clear role for this signalling factor in tumour growth and proliferation.

In this present study, we aim to elucidate the role of both the NFκB and MAPK signalling pathways in mediating TNFα stimulated PI.4-specific *CYP19A1* transcription via the EGR transcription factors. We demonstrate for the first time a direct role for both these signalling pathways in mediating *EGR2* and *EGR3* transactivation leading to PI.4 specific transcription, suggesting that targeted drug therapies directed against these often hyperactivated pathways in cancer may also contribute to inhibiting local estrogen production in the breast.

2. Materials and methods

2.1. Plasmids

NFκB-RE and pTAL constructs were provided by Dr. Simon Chu and have been previously described [17].

2.2. Cell culture

BAFs were isolated from breast reduction mammoplasty by collagenase digestion and cultured as previously described [18]. To stimulate TNFα-dependent pathways, BAFs were grown to ~60% confluency then incubated in serum-free media containing 0.1% BSA (Sigma) for 24 h. Serum-free media containing 5 ng/ml TNFα (Sigma) was then added to cells. For activation of PI.4, 250 nM dexamethasone was added in conjunction with TNFα to serum-free media for 24 h. To inhibit NFκB signalling, BAY-11-7082 (Calbiochem) was added to the culture medium at the specified dose. To inhibit MAPK signalling PD98059, SB203580, SB202190, SP600125 or U0126 (Cell Signalling) was added to the culture medium at the specified dose.

2.3. Transient transfection and reporter assay

BAFs were transiently transfected using electroporation (Nucleofector kit V, Amaxa Biosystems, Lonza, Walkersville, MD, USA) using cell line solution V and pre-adipocyte program T-030. β-galactosidase was transfected as an internal control vector. Cells were incubated for 24 h, then vehicle or TNFα treatment was applied for 24 h. Firefly luciferase activity was measured using the Luciferase Assay System (Promega) and β-galactosidase activity was measured by β-galactosidase assay (Promega).

2.4. Quantitative real time PCR (qRT-PCR)

Total RNA was isolated from cells using the RNeasy Mini kit (QIAGEN). cDNA synthesis was performed on a minimum 200 ng of total RNA using avian myeloblastosis virus reverse transcriptase (Promega) primed by oligo dT or random primers. qRT-PCR of *EGR2* and *EGR3* transcripts was performed using SYBR green detection on the ABI 7900T sequence detection system. *18S* transcripts were detected as an internal RNA loading control. Detection of total *CYP19A1* transcripts was performed on the Roche LightCycler System (Roche Diagnostics) using Fast Start Master SYBR Green I or the ABI 7900T sequence detection system using the *CYP19A1* TaqMan Gene Expression assay (Hs00903413_m1) (Applied Biosystems). PI.4 transcripts were detected on the Roche LightCycler System using Fast Start Master SYBR Green I. Primer sequences are as follows: *EGR2* (sense, TTGACCAGATGAACGGAGTG; anti-sense, GTTGAAGCTGGGGAAGTGAC); *EGR3* (sense, CAATCTGTACCCCGAGGAGA; anti-sense, GGAAGGAGCCGGAGTAAGAG); total *CYP19A1* (sense, TTGGAAATGCTGAACCCGAT; anti-sense, CAGGAATCTGCCGTGGGGAT); PI.4 (sense, GTAGAACGTGACCAACTGG; anti-sense, CACCCGGTTGTAGTAGTTGCAGGCACTGCC); and *18S* (sense, CGGCTACCACATCCAAGGA; anti-sense, GCTGGAATTACCGCGGCT).

3. Results

3.1. The NFkB pathway is constitutively active in BAFs

To determine whether NFκB signalling is active, a luciferase reporter construct under the regulation of a consensus NFκB responsive element motif was transfected into BAFs. Compared to the enhancer-less empty vector control pTAL, the NFκB-RE reporter showed a 6-fold increase in activity under basal conditions. When BAFs were treated with 5 ng/ml TNFα following transfection, this induction was significantly further increased (Fig. 1). This indicates that NFκB signalling is constitutively active in BAFs, and that TNFα further stimulates its activity.

3.2. Inhibition of the NFκB pathway decreases CYP19A1 transcript via PI.4

Having established that NFκB signalling is active in BAFs, an inhibitor of the NFκB pathway was used to determine its role in aromatase expression. BAY-11-7082 is a chemical inhibitor that prevents the activation of the IKK kinase complex. BAFs treated with TNFα/DEX together with BAY-11-7082 at concentrations of 0.5 μM and 1 μM for a period of 24 h showed significantly lower

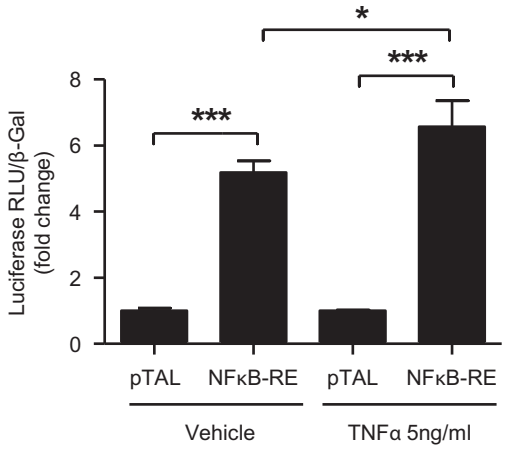

Fig. 1. NFκB is constitutively active in primary human BAFs. BAFs were transfected with either the empty vector reporter pTAL or a reporter construct containing repeating NFκB response elements (NFκB-RE). Following transfection, cells were treated with vehicle control or 5 ng/ml TNFα for 24 h prior to luciferase activity being assessed. Data obtained from three independent experiments. Results are expressed as mean luciferase activity in relative light units (RLU) normalised to β-galactosidase activity, with error bars representing standard error of means. Data was analysed by independent T-test of grouped means ($^{*}p < 0.05$, $^{***}p < 0.001$).

levels of total *CYP19A1* transcripts compared to TNFα/DEX alone (Fig. 2A). This was mirrored by decreases in PI.4 transcripts (Fig. 2B). Higher doses proved toxic to cells after 24 h of treatment.

3.3. Decrease in EGR2 and EGR3 transcript leads to decreased CYP19A1 transcript in response to NFkB inhibition

EGR transcription factors are involved in TNFα-induced *CYP19A1* expression [9]. To determine the role of NKκB in EGR signalling, BAFs were treated for 1 h with BAY-11-7082. *EGR2* expression was dose-dependently inhibited by NFκB inhibition, falling below basal levels at 10 μM BAY-11-7082 (Fig. 2C). Similar effects were seen on *EGR3* mRNA (Fig. 2D), indicating that early loss of EGR transcription factors through NFκB pathway inhibition may result in a decreased capacity to transcribe *CYP19A1*. Alhough higher doses of BAY-11-7082 were toxic in BAFs treated for 24 h, viability was not affected during the shorter 1 h treatment period.

3.4. MAPK signalling is also upstream of EGR2 and EGR3 transcription in response to TNFα

The MAPK signalling cascade is also activated by TNFα binding to its receptor. The effects of two inhibitors targetting distinct branches of the MAPK pathway were examined. SP600125 – which inhibits c-Jun, JNK1,2,3 – showed a dose dependent effect on the reduced expression of *EGR2*, however no significant effect was seen until higher doses (20 μM and 50 μM) of inhibitor were used (Fig. 3A). However when U0126 – inhibitor of MEK1/2 – was added in conjunction with TNFα, the effect on EGR2 expression was immediate from 1 μM dose (Fig. 3B). Conversely, TNFα-induced EGR3 expression showed a more dose-dependent decrease in response to co-treatment with SP600125, with significant reductions observed from 1 μM (Fig. 3C). Again, it appears that U0126 has a more potent effect than SP600125 on EGR3 expression levels, with complete abolishment of TNFα-induced expression at 1 μM. This trend is continued at higher doses, with EGR3 reduced to below basal levels at higher concentrations (Fig. 3D). Other MAPK inhibitors PD98059, SB203580 and SB202190 were also used to test the response of EGR2 and EGR3 transcript to other MAPK inhibitors. All drugs led to a down-regulation of EGR2 and EGR3 transcript in BAFs to differing degrees (Supplementary Fig. 1).

3.5. Inhibition of MAPK signalling decreases expression of CYP19A1 via PI.4

Inhibition of MAPK signalling resulted in down regulation of *EGR2* and *EGR3* transcription in a similar fashion to inhibition of NFκB signalling. Therefore, to determine if MAPK signalling is required for *CYP19A1* expression via PI.4, we treated BAFs with TNFα and Dexamethasone, and co-treated with SP60025 and U0126. Treatment with SP60025 had no effect on total *CYP19A1* transcripts; however inhibition with U0126 significantly down regulated total *CYP19A1* compared to TNFα and DEX treatment alone (Fig. 3E). A similar pattern of promoter I.4-specific transcript levels was observed (Fig. 3F). Inhibition with SP60025 has no effect on PI.4 transcripts, however significant decrease compared to TNFα and DEX alone was observed when cells were co-treated with U0126.

4. Discussion

The signalling pathways downstream of TNFα which lead to transcriptional activation of PI.4 in BAFs are not fully understood. We have shown that both NFκB and MAPK pathways are

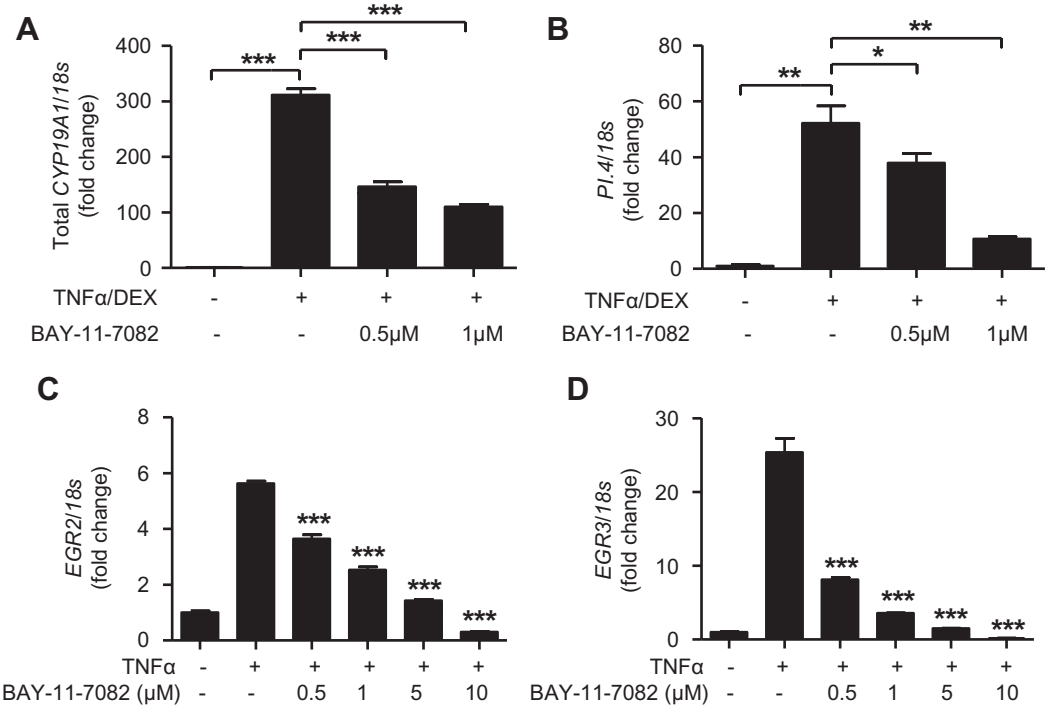

Fig. 2. NFκB inhibition dose dependently decreases *CYP19A1* transcript via PI.4, via early inhibition of EGR2 and EGR3. BAFs were treated for 24 h with TNFα (5 ng/ml) and DEX (250 nM), with or without the NFκB pathway inhibitor BAY-11-7082 (0.5 μM or 1 μM). mRNA expression of (A) *CYP19A1* total and (B) PI.4-specific transcripts were then detected. (C) BAFs were treated for 1 h with TNFα (5 ng/ml) and BAY-11-7082 at varying concentrations. qRT-PCR was then used to detect transcript levels of EGR2 and (D) EGR3. Data obtained from three independent experiments performed in triplicate. Error bars represent standard error of means, data analysed by one-way ANOVA with a Newman–Keuls post-test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

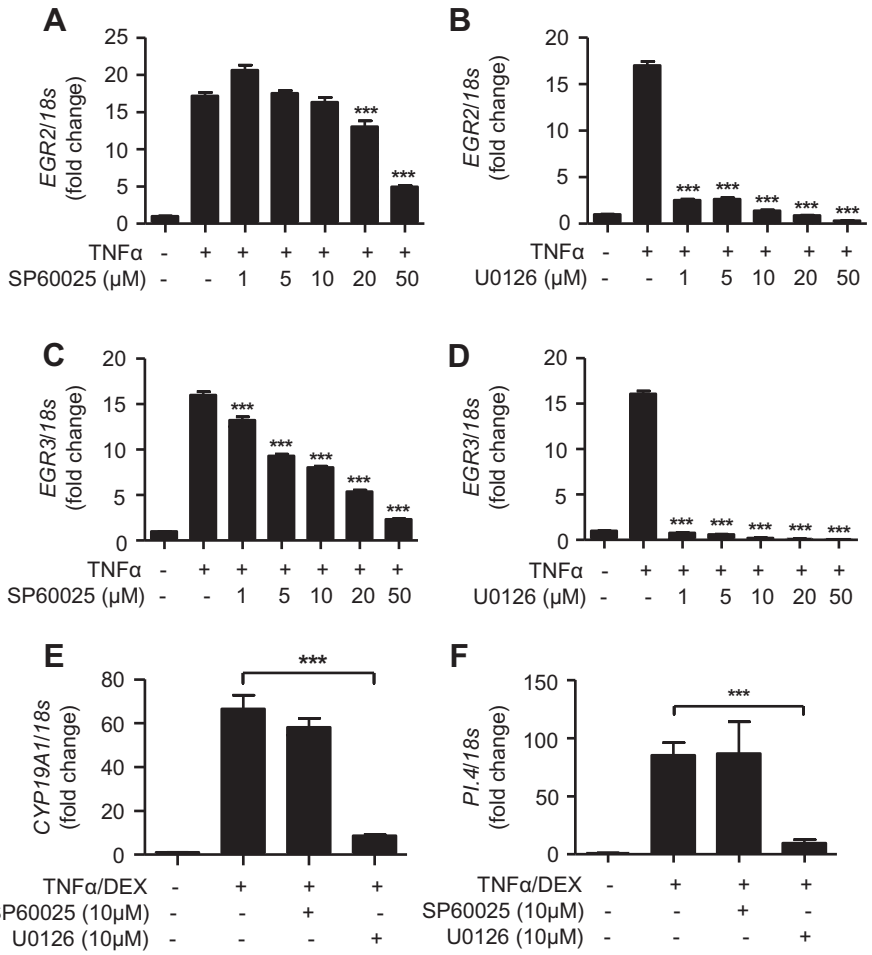

Fig. 3. Inhibition of MEK1/2 decreases EGR2 and EGR3 expression, leading to a decrease in CYP19A1 mRNA via PI.4. BAFs were pre-treated with MAPK pathway inhibitors for 30 min, then treated for 1 h with TNFα (5 ng/ml). qRT-PCR was then used to detect transcript levels of (A and B) EGR2 and (C and D) EGR3. BAFs were treated with TNFα (5 ng/ml) and DEX (250 nM) together with either SP60025 (5 μM) or U0126 (5 μM) for 24 h. Transcript levels of total (E) *CYP19A1* or (F) PI.4 specific transcripts were then detected by qRT-PCR. Data obtained from three independent experiments performed in triplicate. Error bars represent standard error of mean, data analysed by one-way ANOVA with a Newman–Keuls post-test (***$p < 0.001$).

downstream of TNFα, leading to transcription of the EGR genes and ultimately *CYP19A1* via the adipose specific promoter PI.4. Inhibition of either NFκB or MAPK signalling leads to decreased expression of *EGR*s and *CYP19A1*.

Little is known about the transcriptional regulation of *EGR2* and *EGR3*, with the majority of information focusing on the initially isolated family member *EGR1*. Given the rapid effects NFκB and MAPK inhibitors on *EGR2* and *EGR3* expression, it is likely there is direct regulation of these two transcription factors by the NFκB and MAPK-induced transcriptional complexes. In T-cells, Vasoactive Intestinal Peptide and pituitary adenylate cyclase-activating polypeptide inhibit nuclear translocation of NFκB and also down regulate expression of *EGR2* and *EGR3*, although no direct link between the two events was established [19]. An analysis of the *EGR2* and *EGR3* regulatory region reveals potential NFκB response elements present in both promoters (data not shown), consistent with a direct interaction. NFκB rapidly induces immediate early response genes following stimulation with TNFα, via a cooperative interaction with Sp1. Transcriptional machinery is constitutively bound to promoters, as mediated by Sp1, and NFκB may then enhance the initiation rate rapidly [20]. Although this was only shown using the representative immediate early gene *A20* in response to TNFα, this is one potential mechanism for rapid transcription of *EGR2* and *EGR3* by NFκB.

EGR transcription showed differential sensitivity to inhibition of different components of the MAPK pathway. Targetting c-Jun and JNK1,2,3 with SP60025 resulted in significant *EGR2* inhibition only at higher 20 μM and 50 μM doses, but a dose-dependent inhibition of *EGR3* expression significant at 1 μM. Conversely, targetting MEK1/2 with U0126 had a much more dramatic effect on EGR transcription. *EGR2* transcripts were reduced to basal levels at 10 μM and below basal levels at higher doses, and *EGR3* transcripts were repressed to basal levels or below immediately from the lowest 1 μM dose used. This suggests a preferential role for MEK1/2 in EGR regulation, versus the c-Jun or JNK component of MAPK signalling. *EGR2* and *EGR3* promoter analysis however does not uncover direct binding sites for any of the inhibited factors. One possible explanation for this is the potential for crosstalk between the NFκB signalling pathway and MAPK signalling components. Several examples of NFκB regulation of MAPK-regulated transcription factors are known, and this may be a mechanism by which inhibition of MAPK leads to a down regulation of *EGR* transcription.

Our results indicate a clear role for NFκB in mediating aromatase induction by TNFα. A number of previous studies have shown a potential role for NFκB signalling in *CYP19A1* transcriptional activation, but our data is provides direct evidence for the first time. A study of primary human endometrial stromal cells found that treatment with the progestin drug dienogest inhibited both NFκB

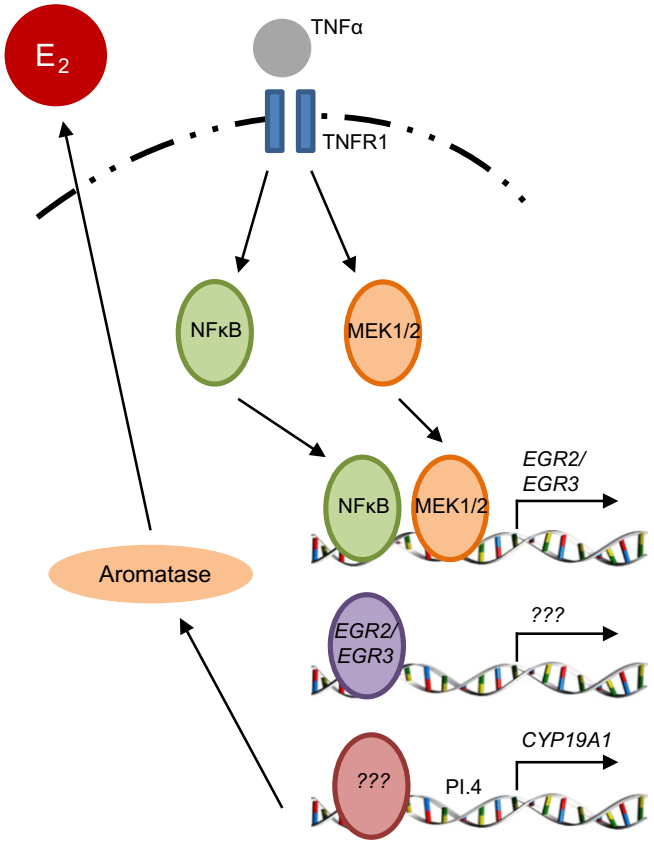

Fig. 4. Model of TNFα induced aromatase expression. In breast adipose fibroblasts, TNFα binds to its receptor TNFR1 to initiate activation of the NFκB and MAPK pathways. NFκB and MEK1/2 lead to transcription of EGR2 and EGR3, which in turn activates *CYP19A1* PI.4 by indirect means of transcribing an intermediate factor.

DNA-binding activity and aromatase expression, suggesting a possible link [21]. In human breast adipose fibroblasts, cell shape-induced aromatase induction can be significantly down regulated by BAY-11-7082. This was independent of the canonical NFκB pathway however, as a specific siRNA directed against the IKKβ component ablated aromatase transcription, but not siRNAs against IKKα or IKK [22]. Increased binding activity of NFκB coupled with increased expression and activity of aromatase is also observed in the inflamed breast adipose tissue of overweight and obese women [23]. Interestingly, NFκB signalling has been implicated in *CYP19A1* transcription via the proximal promoter II, commonly upregulated in breast cancer, with a study in ovarian granulosa tumour cell lines showing direct binding of the p65 subunit to PII, and NFκB inhibitors decreasing PII-driven luciferase response [24]. Our findings suggest that PI.4 may also be downstream of NFκB activation. Despite clear roles of NFκB in breast cancer proliferation and aromatase activation, clinical use of NFkB inhibitors is precluded by adverse effects due to global inhibition of NFκB signalling [25].

MAPK signalling also appears to be downstream of TNFα stimulation, and EGR/PI.4 activation appears to be mediated more specifically by MEK1/2. There are currently conflicting reports within published literature pertaining to role of MAPK in aromatase induction. In human breast adipose fibroblasts, downstream of PGE2 and PKA/PKC activation, p38/MAPK can phosphorylate ATF-2 and c-Jun, which subsequently interact with and upregulate *CYP19A1* PII [26]. Conversely, active MAPK signalling downregulates aromatase transcript and therefore estrogen production in immature sertoli cells [27]. Pharmacologically targetting MEK1/2 presents an attractive option, given its involvement in numerous cancer processes. A number of MEK1/2 inhibitors are currently at the stage of clinical trials, though there is yet to be one developed for full clinical use [28]. Early indicators suggest that they can cause regression of tumours *in vivo* including colorectal, thyroid and myeloma [29–31], although adverse side effects are compounding clinical trials. Our research suggests that with further development of specific MEK1/2 inhibitors, ER+ breast cancer may be amongst carcinomas that may be treated.

In conclusion, we have elucidated the downstream signalling pathways involved in TNFα induced aromatase transcription via EGR2 and EGR3 (Fig. 4). The data presented here represents a significant development in understanding local estrogen production in the breast, and offers new avenues by which therapeutic intervention of ER+ breast cancer may be further developed.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.02.058.

References

[1] L. Binkhorst, T. van Gelder, R.H. Mathijssen, Individualization of tamoxifen treatment for breast carcinoma, Clin. Pharmacol. Ther. 92 (4) (2012) 431–433.
[2] E.R. Simpson, Y. Zhao, V.R. Agarwal, M.D. Michael, S.E. Bulun, M.M. Hinshelwood, S. Graham-Lorence, T. Sun, C.R. Fisher, K. Qin, C.R. Mendelson, Aromatase expression in health and disease, Recent Prog. Horm. Res. 52 (1997) 185–213. discussion 213–214.
[3] N. Harada, K. Yamada, K. Saito, N. Kibe, S. Dohmae, Y. Takagi, Structural characterization of the human estrogen synthetase (aromatase) gene, Biochem. Biophys. Res. Commun. 166 (1) (1990) 365–372.
[4] N. Harada, T. Utsumi, Y. Takagi, Tissue-specific expression of the human aromatase cytochrome P-450 gene by alternative use of multiple exons 1 and promoters, and switching of tissue-specific exons 1 in carcinogenesis, Proc. Natl. Acad. Sci. USA 90 (23) (1993) 11312–11316.
[5] M.S. Mahendroo, C.R. Mendelson, E.R. Simpson, Tissue-specific and hormonally controlled alternative promoters regulate aromatase cytochrome P450 gene expression in human adipose tissue, J. Biol. Chem. 268 (26) (1993) 19463–19470.
[6] Y. Zhao, C.R. Mendelson, E.R. Simpson, Characterization of the sequences of the human CYP19 (aromatase) gene that mediate regulation by glucocorticoids in adipose stromal cells and fetal hepatocytes, Mol. Endocrinol. 9 (3) (1995) 340–349.
[7] Y. Zhao, V.R. Agarwal, C.R. Mendelson, E.R. Simpson, Transcriptional regulation of CYP19 gene (aromatase) expression in adipose stromal cells in primary culture, J. Steroid Biochem. Mol. Biol. 61 (3–6) (1997) 203–210.
[8] Y. Zhao, J.E. Nichols, R. Valdez, C.R. Mendelson, E.R. Simpson, Tumor necrosis factor-alpha stimulates aromatase gene expression in human adipose stromal cells through use of an activating protein-1 binding site upstream of promoter 1.4, Mol. Endocrinol. 10 (11) (1996) 1350–1357.
[9] S.Q. To, E.R. Simpson, K.C. Knower, C.D. Clyne, Involvement of early growth response factors in TNFalpha-induced aromatase expression in breast adipose, Breast Cancer Res. Treat. 138 (1) (2013) 193–203.
[10] A. Inoue, Y. Omoto, Y. Yamaguchi, R. Kiyama, S.I. Hayashi, Transcription factor EGR3 is involved in the estrogen-signalling pathway in breast cancer cells, J. Mol. Endocrinol. 32 (3) (2004) 649–661.
[11] K. Shostak, A. Chariot, NF-kappaB, stem cells and breast cancer: the links get stronger, Breast Cancer Res. 13 (4) (2011) 214.
[12] P.C. Cogswell, D.C. Guttridge, W.K. Funkhouser, A.S. Baldwin Jr., Selective activation of NF-kappa B subunits in human breast cancer: potential roles for NF-kappa B2/p52 and for Bcl-3, Oncogene 19 (9) (2000) 1123–1131.
[13] Q. Yu, Y. Geng, P. Sicinski, Specific protection against breast cancers by cyclin D1 ablation, Nature 411 (6841) (2001) 1017–1021.
[14] M.A. Rivas, R.P. Carnevale, C.J. Proietti, C. Rosemblit, W. Beguelin, M. Salatino, E.H. Charreau, I. Frahm, S. Sapia, P. Brouckaert, P.V. Elizalde, R. Schillaci, TNF alpha acting on TNFR1 promotes breast cancer growth via p42/P44 MAPK, JNK,

Akt and NF-kappa B-dependent pathways, Exp. Cell Res. 314 (3) (2008) 509–529.
[15] F. Meng, H. Zhang, G. Liu, B. Kreike, W. Chen, S. Sethi, F.R. Miller, G. Wu, P38gamma mitogen-activated protein kinase contributes to oncogenic properties maintenance and resistance to poly (ADP-ribose)-polymerase-1 inhibition in breast cancer, Neoplasia 13 (5) (2011) 472–482.
[16] J.W. Antoon, M.R. Bratton, L.M. Guillot, S. Wadsworth, V.A. Salvo, S. Elliott, J.A. McLachlan, M.E. Burow, Pharmacology and anti-tumor activity of RWJ67657, a novel inhibitor of p38 mitogen activated protein kinase, Am. J. Cancer Res. 2 (4) (2012) 446–458.
[17] S. Chu, Y. Nishi, T. Yanase, H. Nawata, P.J. Fuller, Transrepression of estrogen receptor beta signalling by nuclear factor-kappab in ovarian granulosa cells, Mol. Endocrinol. 18 (8) (2004) 1919–1928.
[18] G.E. Ackerman, M.E. Smith, C.R. Mendelson, P.C. MacDonald, E.R. Simpson, Aromatization of androstenedione by human adipose tissue stromal cells in monolayer culture, J. Clin. Endocrinol. Metab. 53 (2) (1981) 412–417.
[19] M. Delgado, D. Ganea, Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit expression of Fas ligand in activated T lymphocytes by regulating c-Myc, NF-kappa B, NF-AT, and early growth factors 2/3, J. Immunol. 166 (2) (2001) 1028–1040.
[20] E. Ainbinder, M. Revach, O. Wolstein, S. Moshonov, N. Diamant, R. Dikstein, Mechanism of rapid transcriptional induction of tumor necrosis factor alpha-responsive genes by NF-kappaB, Mol. Cell. Biol. 22 (18) (2002) 6354–6362.
[21] K. Yamanaka, B. Xu, I. Suganuma, I. Kusuki, S. Mita, Y. Shimizu, K. Mizuguchi, J. Kitawaki, Dienogest inhibits aromatase and cyclooxygenase-2 expression and prostaglandin E(2) production in human endometriotic stromal cells in spheroid culture, Fertil. Steril. 97 (2) (2012) 477–482.
[22] S. Ghosh, A. Choudary, N. Musi, Y. Hu, R. Li, IKKbeta mediates cell shape-induced aromatase expression and estrogen biosynthesis in adipose stromal cells, Mol. Endocrinol. 23 (5) (2009) 662–670.
[23] P.G. Morris, C.A. Hudis, D. Giri, M. Morrow, D.J. Falcone, X.K. Zhou, B. Du, E. Brogi, C.B. Crawford, L. Kopelovich, K. Subbaramaiah, A.J. Dannenberg, Inflammation and increased aromatase expression occur in the breast tissue of obese women with breast cancer, Cancer Prev. Res. (Phila) 4 (7) (2011) 1021–1029.
[24] W. Fan, T. Yanase, H. Morinaga, Y.M. Mu, M. Nomura, T. Okabe, K. Goto, N. Harada, H. Nawata, Activation of peroxisome proliferator-activated receptor-gamma and retinoid X receptor inhibits aromatase transcription via nuclear factor-kappaB, Endocrinology 146 (1) (2005) 85–92.
[25] J. Ling, R. Kumar, Crosstalk between NFkB and glucocorticoid signalling: a potential target of breast cancer therapy, Cancer Lett. 322 (2) (2012) 119–126.
[26] D. Chen, S. Reierstad, Z. Lin, M. Lu, C. Brooks, N. Li, J. Innes, S.E. Bulun, Prostaglandin E(2) induces breast cancer related aromatase promoters via activation of p38 and c-Jun NH(2)-terminal kinase in adipose fibroblasts, Cancer Res. 67 (18) (2007) 8914–8922.
[27] C.A. McDonald, A.C. Millena, S. Reddy, S. Finlay, J. Vizcarra, S.A. Khan, J.S. Davis, Follicle-stimulating hormone-induced aromatase in immature rat Sertoli cells requires an active phosphatidylinositol 3-kinase pathway and is inhibited via the mitogen-activated protein kinase signalling pathway, Mol. Endocrinol. 20 (3) (2006) 608–618.
[28] C. Fremin, S. Meloche, From basic research to clinical development of MEK1/2 inhibitors for cancer therapy, J. Hematol. Oncol. 3 (2010) 8.
[29] Y.C. Henderson, Y. Chen, M.J. Frederick, S.Y. Lai, G.L. Clayman, MEK inhibitor PD0325901 significantly reduces the growth of papillary thyroid carcinoma cells *in vitro* and *in vivo*, Mol. Cancer Ther. 9 (7) (2010) 1968–1976.
[30] T. Yamaguchi, R. Kakefuda, N. Tajima, Y. Sowa, T. Sakai, Antitumor activities of JTP-74057 (GSK1120212), a novel MEK1/2 inhibitor, on colorectal cancer cell lines *in vitro* and *in vivo*, Int. J. Oncol. 39 (1) (2011) 23–31.
[31] K. Kim, S.Y. Kong, M. Fulciniti, X. Li, W. Song, S. Nahar, P. Burger, M.J. Rumizen, K. Podar, D. Chauhan, T. Hideshima, N.C. Munshi, P. Richardson, A. Clark, J. Ogden, A. Goutopoulos, L. Rastelli, K.C. Anderson, Y.T. Tai, Blockade of the MEK/ERK signalling cascade by AS703026, a novel selective MEK1/2 inhibitor, induces pleiotropic anti-myeloma activity *in vitro* and *in vivo*, Br. J. Haematol. 149 (4) (2010) 537–549.