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RESEARCH ARTICLE

5a-dihydrotestosterone (DHT) inhibits Tumor Necrosis Factor - alpha (TNF-a) induced Janus Kinase-1 (JNK-1) activation and apoptosis in breast cancer in p21 dependent manner

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ABSTRACT

Beside established primary reproductive functions, the male hormone androgen act as a growth and survival factor for most of its target tissues. Though male androgenic hormones are widely studied, still some aspects of underlying mechanisms are not completely clear. In addition to their influence on female reproductive hormones which are considered to be crucial factor in breast carcinogenesis, several reports shows that male androgenic hormones may have direct influences on breast cancer tissues. Here, we have used dihydrosterone as model of male androgen to understand its impact on some major molecular circuitry and their by in the process of breast carcinogenesis. We observed that DHT convincingly inhibits tumor necrosis factor-alpha (TNF-α) induced Janus Kinase-1 (JNK-1) activation and apoptosis in breast cancer cells, MCF-7 in p21 dependent manner. Inhibition by androgen requires the transcription activity of androgen receptor (AR) and de novo protein synthesis. AndrogenAR induces expression of p21 that in turn inhibits tumor necrosis factor-α induced JNK activation and apoptosis. Our results reveal a novel cross-talk between AR and JNK in breast cancer (MCF-7) cells, thereby providing a molecular mechanism underlying the survival function of androgen on its target tissues or organ.

KEYWORDS: Tumor Necrosis Factor - alpha (TNF-á), Janus Kinase-1 (JNK-1) ,Breast cancer, Apoptosis

INTRODUCTION

Androgens are steroid hormones that have pleiotropic functions in androgen-responsive tissues or organs and act as latent transcription factor. Upon ligand binding the androgen receptor (AR) molecules get dimerized and translocate to nucleus. Inside the nucleus, these androgen receptor dimer bind to specific target sequence called androgen response element (ARE) to induce expression of its target genes regulating diverse cellular activities ranging from proliferation to apoptosis[1-3]. Several studies have clearly established that AR have important role in normal development of the breast tissue[4]. At the time of puberty, normal mammary tissue expresses AR in 20 % of the total cells which is higher than estrogen receptor (ER) (10 %). AR is considered to be responsible for the maintenance of ER-induced cell proliferation and normal development of breast[5]. During this phase, AR activity balances E2-induced mammary cell proliferation ensuring correct development of the gland [4, 6, 7]. Interestingly, AR is expressed in 70-90% of breast cancer cases and have been investigated for its role in progression of breast cancer [8-15]. AR

expression in breast cancer is viewed to be associated with tumor responsiveness, lower tumor burden and favourable differentiation. Possible role of androgen-mediated mechanism is also supported by reports estimating high local expression of 5á-dihydrotestosterone (DHT) and its regulation by local aromatase in breast tumor tissue compared to peripheral plasma level.

Previous studies in prostate cancer suggested some steroids such as corticosol, R1881 and DHT inhibited the TNF alpha induced apoptosis in prostate cancer[16].The pro-inflammatory cytokine tumor necrosis factor alpha (TNF-á) regulates immune responses, inflammation, and programmed cell death (apoptosis)[17]**.** TNF-á exerts its biological activities by binding to type 1 and type 2 receptors activating multiple signaling pathways, including IkB kinase (IKK), C-Jun N-terminal protein kinase (JNK), and caspases[17-19]**.** C-Jun N-terminal kinase (JNK) also known as stress-activated protein kinase (SAPK), is a subfamily of the mitogen-activated protein kinase (MAPK) super family[20-22].

The JNK family has three isoforms: JNK1 and JNK2 and JNK3[23-25]. JNK can be activated by various extracellular stimuli, from pro-inflammatory cytokines such as tumor necrosis factor (TNF-á) to environmental stress like UV light[26]. Activated JNK can regulate the activity of several transcription factors, such as C-Jun, ATF-2, Elk-1, p53, and c-Myc, or non-transcription factors, including members of the Bcl-2 family[23, 25-27]. The activity of JNK can be regulated by protein phosphatases, NFkB, and scaffold proteins such as JIP, â-arrestin, and JSAP1[24, 25]. Accumulating evidence suggest that JNK plays a critical role in regulation of many fundamental cellular activities, including apoptosis [23, 25].

TNF-á type 1 death receptor domain interact with the intracellular proteins that activates JNK[28]. IKK activation inhibits apoptosis through the transcription factor NF-kB whose target genes include those that encode inhibitors of both caspases and JNK. Despite activation of the anti-apoptotic IKK/NF-kB pathway, TNF-á is able to induce apoptosis in cells sensitive to it, such as human breast carcinoma MCF-7 and mouse fibroblast LM cells**.** TNF-á induced apoptosis is suppressed by inhibition of the JNK pathway but promoted by its activation. Furthermore, activation of JNK by TNF-á was transient in TNF-á insensitive cells but prolonged in sensitive cells. Conversion of JNK activation from prolonged to transient suppressed TNF-á-induced apoptosis. Thus, absence of NF-kB-mediated inhibition of JNK activation contributes to TNF-á-induced apoptosis.In present study we have studied (why you have decided to study? Write briefly before this sentence)the effect of DHT on TNF-á induced JNK activation and apoptosis in breast cancer (MCF-7) cells and elucidate its mechanism of action.

EXPERIMENTAL PROCEDURE

Reagents and antibodies: All reagents and primers were procured from Sigma Aldrich, USA. Antibodies and kinase assay kit were procured from cell signalling technology (CST), USA.

Cell culture and cell lines: A Breast cancer cell line MCF-7 cell procured from American type cell collection (ATCC) and was cultured in RPMI medium supplemented with 10% fetal bovine serum and cells were maintained in CO2 incubator at 5% CO2 under humid condition.

Anti-Proliferative assay: MTT assay was used to analyse the anti-proliferative activity of TNF-á in MCF-7 cells. Briefly $1X10^4$cells were seeded in 96 well plates and allowed to grow for 24h. Cells were treated with different concentration of TNF-á and DHT either alone or in combination. At the end of treatment, 20µl MTT (5mg/mL) were added into each well and incubated for 3h. Formazan crystals formed were dissolved in 200 µl DMSO and OD were

recorded at 540 nm and IC50 were calculated by using Prism 3 (Version 3.1).

Apoptotic assay: TNF-á induced apoptosis were analysed by using AnnexinV/PI staining using flow cytometry. MCF-7 cells ($1X10^5$) were seeded in six well plates and allowed to grow for 24h and subsequently cells were treated with TNF-á (20 ng/mL) and DHT (50 nM) either alone or in combination of both, for 32h and at the end of incubation cells were harvested after mild trypsiniation and were re-suspended in 1X binding buffer. Cells were stained with AnnexinV/PI for 10 minutes and samples were acquired by flow-cytometer.

Immune Complex Kinase (JNK kinase activity) Assay and Immuno-blotting: Cells were harvested and lysed in RIPA buffer in the presence of protease and phosphatise inhibitors cocktails. Protein was estimated using Bradford reagent. For western blotting 40µg protein were loaded and immune complex kinase assay was performed as according to manufactures instructions.

Brifely, 500µg protein was incubated with pJNK tagged sepharose beads for overnight at 4^0C followed by washing with 1X cell lysis buffer and kinase buffer. Kinase reaction were set up in 50µL and incubated at 30^0C for 30 minutes. At the end of incubation reaction was terminated by adding 25µl 3X protein loading dye. 20µl samples were loaded on 10 % SDS-PAGE from each sample and electrophoreses. After running gel protein were transfer to PVDF membrane and blocked with 1% BSA and incubated with specific primary antibodies pC-Jun and C-Jun (JNK kinase assay) and pJNK, JNK, pC-JUN, C-JUN, p21 and â-actin (IB), for overnight then washed with 1XTBST and again incubated with corresponding secondary antibodies and washed again and Develops using HRP-substrate ECL using chemidoc.

RESULTS

DHT inhibits the TNF alpha induced apoptosis via converting prolong to transient JNK activation: The male androgen hormone is essential for the growth and survival of its target tissues or organ [1, 29]. However, the underlying mechanism is incompletely understood. To address this issue, we tested the effect of androgen on TNF-á induced cell death in breast cancer cells (MCF-7). MCF-7 cells are TNF-á sensitive cell which induced apoptosis in response to TNF-á treatment. We have first assessed the effect of DHT on the TNF-á induced loss of cell viability by MTT assay. MCF-7 cells were treated with different concentrations of TNF-á alone on in the presence of different concentration of DHT for 48h. TNF-á induced the loss of cell viability in dose dependent manner. Our results suggested 50 nM and 100 nM DHT significantly inhibited the TNF-á induced loss of cell viability in all three concentration of TNF-á (**Figure 1).**

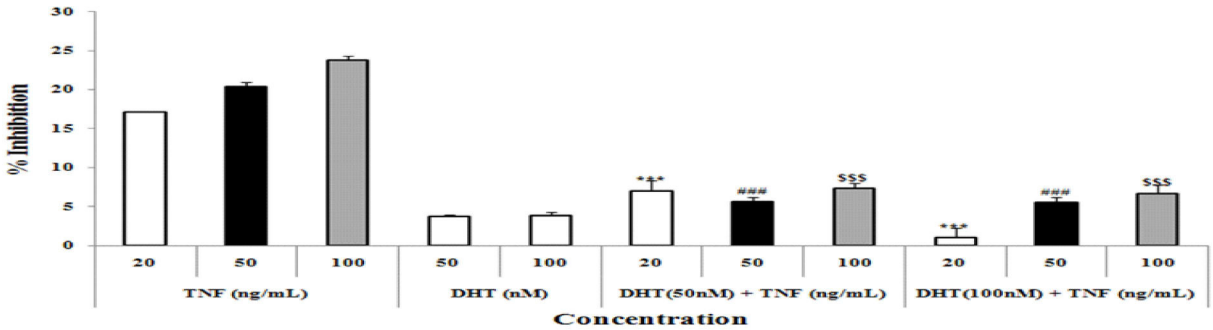

FIGURE 1: **DHT inhibits TNF-α induced loss of cell viability:** MCF-7 cells were treated with different concentrations of TNF-α and DHT for 48h at the end of incubation 20µl MTT was added to each and OD was recorded and % inhibition were calculated**.**

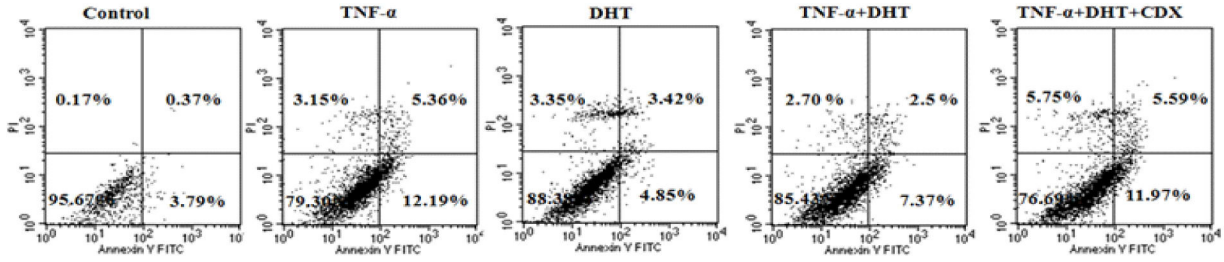

FIGURE 2: **DHT inhibits the TNF-α induced apoptosis**: MCF-7 cells were treated with TNF-α alone or in the presence of DHT and cells were stained with AnnexinV/PI.

We have also check the effect of DHT on the TNF-á induced apoptosis flow-cytometrically staining the cells with AnnexinV/PI results suggested that DHT inhibits the TNF-á induced apoptosis which get rescued in the presence of androgen receptor antagonist bicalutamide (CDX) suggesting that this effect may AR dependent (**Figure 2**).

Because prolonged JNK1 activation is essential for TNF-á to induce apoptosis in the absence of NF-kB activation [23, 30], we were curious whether DHT inhibits prolonged JNK1 activation. To test this hypothesis MCF-7 cells were pre-treated with control ethanol and DHT for 1h followed by stimulation with TNF-á treatment for different time points. Immune complex kinase assay and immuno-blots results suggested that TNF-á induced JNK activation was prolonged in cells pre-treated with ethanol (**Figure 3**) while it is transient in DHT pre-treated MCF-7 cells (**Figure 4**). Under these conditions DHT pre-treatment also significantly inhibits the JNK target genes pC-Jun.

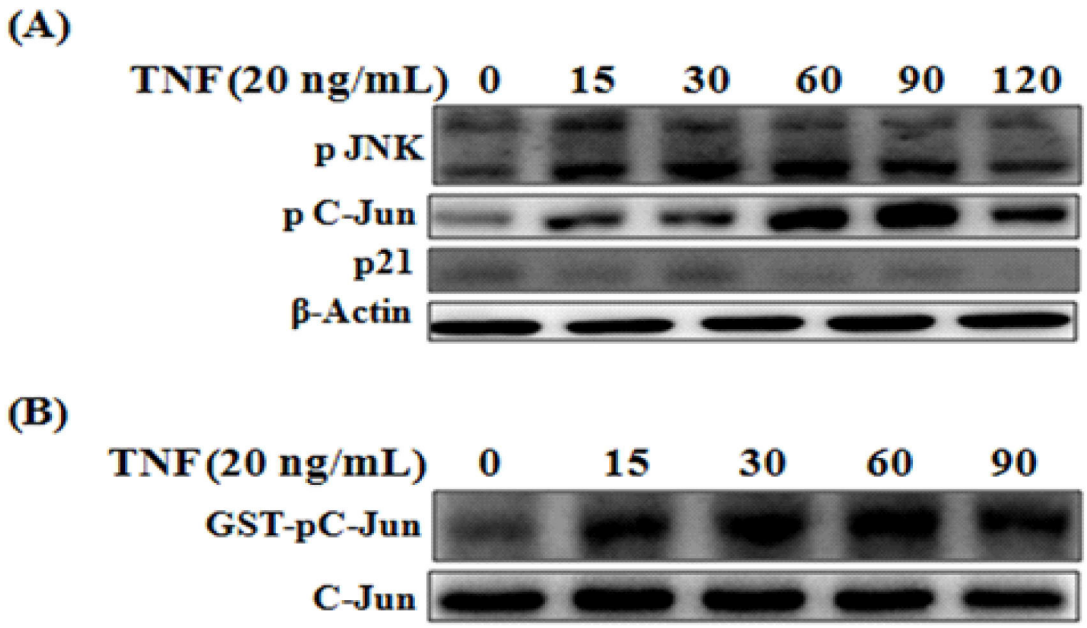

FIGURE 3: TNF-α induced the prolong activation of JNK and its target gene in breast cancer cells: MCF-7 cells were treated with TNF-α (20ng/mL) for different time points and cell lysate probed with pJNK, pC-Jun, p21 (a) Immuno-blot (b) JNK kinase assay**.**

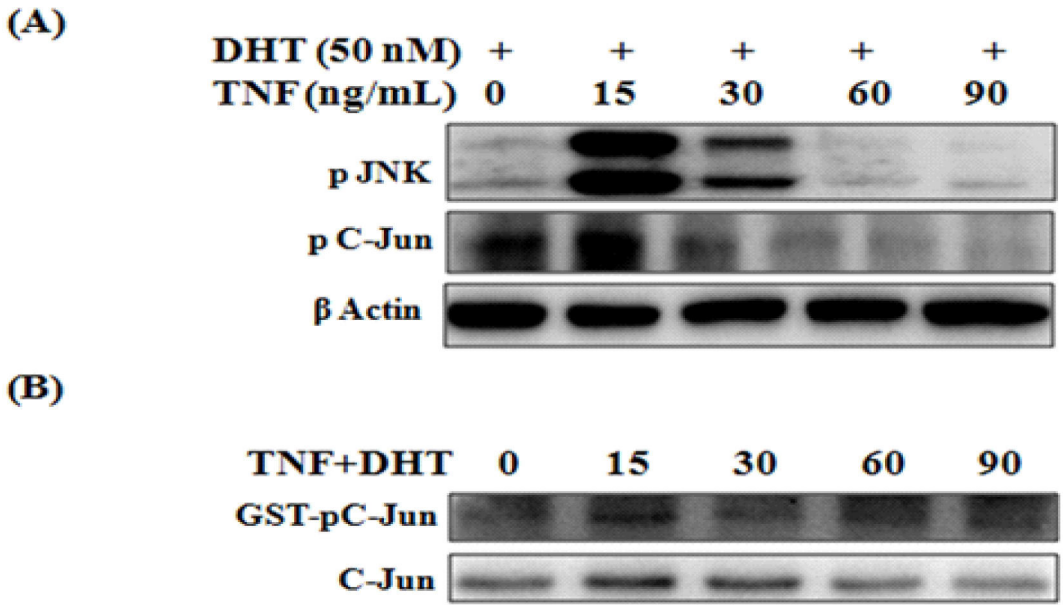

FIGURE 4: DHT inhibits TNF-α induced apoptosis by inhibiting the prolong activation of the JNK: MCF-7cells were pre-treated with DHT (50nM, 60 minutes) followed by treatment with TNF-α (20ng/mL) for 15 minutes and cell lysate was probed with pJNK, pC-Jun, C-Jun antibodies (a) Immuno-blot (b) JNK kinase assay.

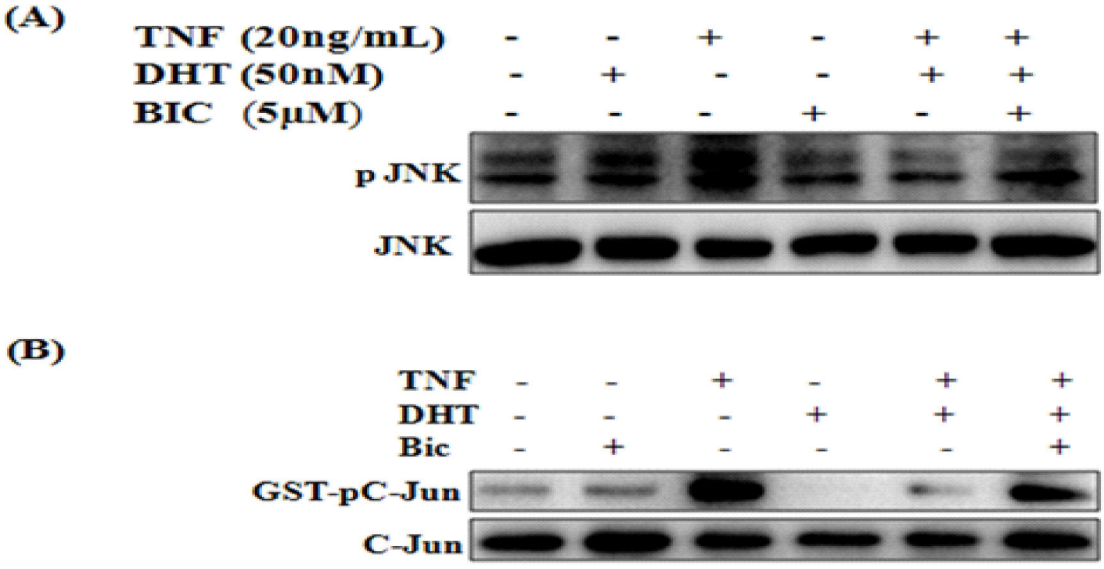

FIGURE 5: AR-dependent transcription is required for androgen to inhibit JNK activation and TNF-α induced apoptosis. MCF-7 were pre-treated with (5μM, 30 min) followed by treatment with or without DHT (50 nM, 90 min). Cells were stimulated with TNF-α (20 ng/ml) for 15 min. (a) JNK activity (KA) and (b) content (IB) were measured.

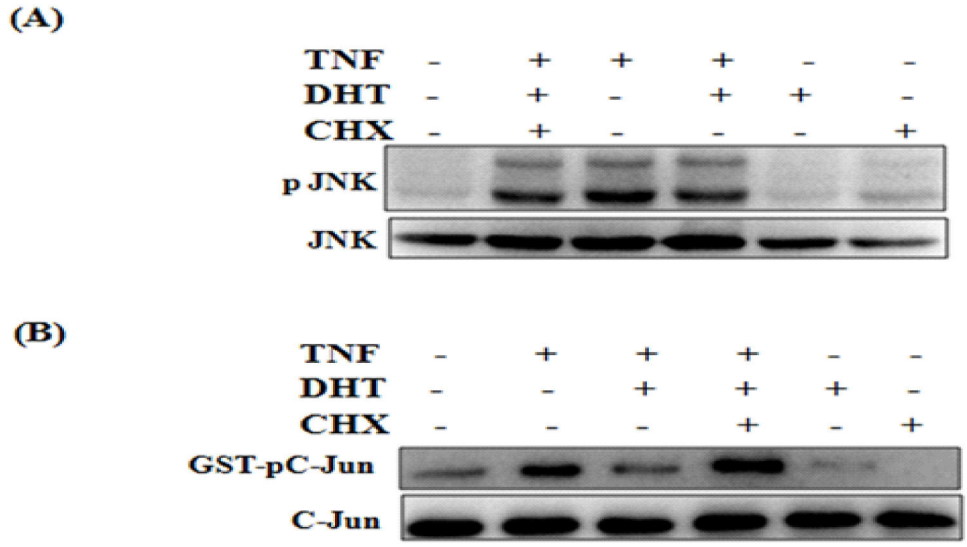

FIGURE 6: AR-dependent transcription is required for androgen to inhibit JNK activation and TNF-α induced apoptosis: MCF-7 were pretreated with CHX (1ng/ml, 30 min) followed by treatment with or without DHT (50 nM, 90 min). Cells were stimulated with TNF-α (20 ng/ml) for 15 min. (a) JNK activity (KA) and (b) content (IB) were measured.

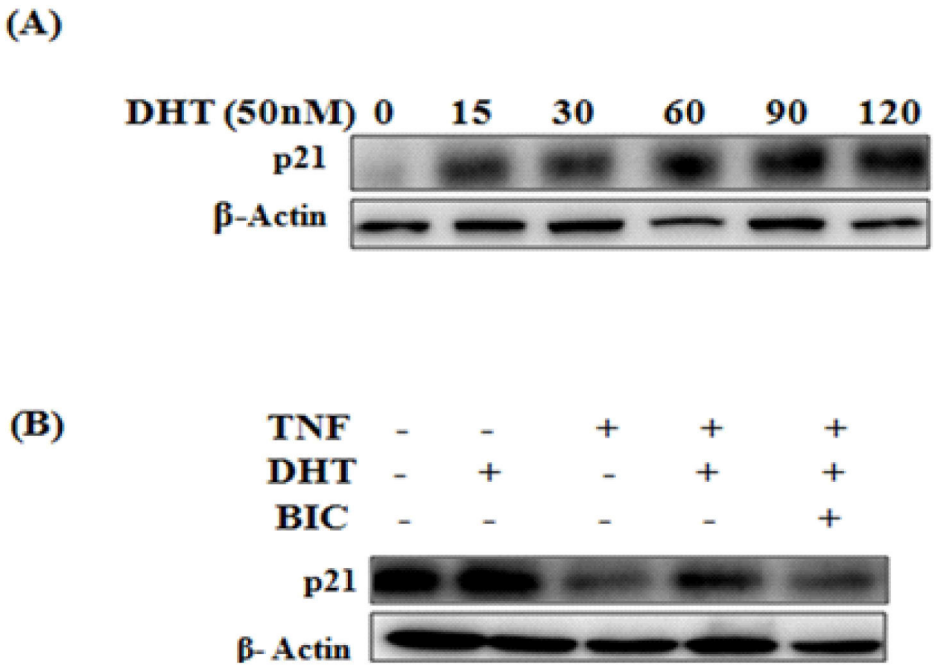

FIGURE 7: Inhibition of TNF-á induced JNK activation by androgen depends on p21. MCF-7 cells were stimulated by DHT (50 nM) or left alone for the times indicated. (a) Expression levels of p21 proteins were analyzed by immunoblotting using anti-p21 antibody. (b) MCF-7cells were treated with bicalutamide (5μM,30 mins) followed by treatment with DHT (50 nM, 90 mins).Cells were stimulated with TNF-á (20 ng/ml) for 15 min. p21 were analyzed by immune-blotting (IB).

This suggests that DHT inhibited TNF-á induced JNK activation occurred at or above JNK activating kinase JNKKs [31, 32], with a delayed kinetics. Collectively, these results demonstrate that androgen (DHT) inhibits TNF-á induced apoptosis through suppression of prolonged JNK1 activation.

Inhibition of TNF-á induced JNK Activation and Apoptosis by Androgen Requires AR

Androgens exert its biological effects via androgen receptors AR that get dimerise upon ligand binding and translocate to nucleus where it binds to ARE and transcribe its target genes which are essential for many cellular functions [29]. To evaluate whether the inhibitory effect of androgen depends upon AR, we used the specific androgen antagonist bicalutamide (CDX) to block AR transcription activity [33].

Immuno blotting and immune complex kinase assays showed that pre-treatment with bicalutamide significantly reduced the inhibitory effect of androgen on JNK activation by TNF-á. Consistently (**Figure 5**), treatment of cells with a protein synthesis inhibitor CHX (Cyclo-hexamide) also abrogated the inhibition by androgen on TNF-á induced JNK activation (**Figure 6**). CHX alone had no detectable effects on JNK activation in In parallel experiments, we found that bicalutamide also abolished the inhibitory effect of androgen on TNF-á induced apoptosis (**Figure 5, 2**). Thus, the inhibitory effect of androgen on TNF-á induced JNK1 activation and apoptosis depends on AR transcription activity and de novo protein synthesis (**Figure 5, 6**).

p^{21} mediates the Inhibition by Androgen on TNF-á induced JNK1 Activation

Previous results suggested inhibition by androgen on TNF-á induced JNK1 activation and apoptosis requires the transcription activity of AR and de novo protein synthesis promoted us to search for androgen-induced JNK inhibitors. It has been reported that p21 is a target gene of AR [34] and that has been shown to inhibit JNK activation [35, 36].

To test this idea, MCF-7 cells were treated with DHT for different time points immune blot results suggested the DHT treatment induces p21 expression time dependently (**Figure 7a**) while TNF-á have no effect on p21 expression (**Figure 3a**). Furthermore the level of p21 is induces in the presence of DHT while it level get diminished in the presence of androgen receptor antagonist, bicalutamide (**Figure 7b**) suggesting the role of p21 in the inhibition of TNF-á induced JNK action and apoptosis.

DISCUSSION

Androgen has pleiotropic role on growth and survival of its target tissues or organs [1, 29]. Recent evidence suggested that androgen receptor (AR) have a role in normal development of the breast tissue [4]. At puberty normal mammary tissue express AR in 20% cells even more than ER (10%). It is responsible for the maintenance of ER-induced cell proliferation and normal development of breast[5]. AR activity is able to balance E2-induced cell proliferation, influencing the correct development of the gland [4, 6, 7].

Furthermore recent evidences suggested that androgen receptor is expressed in 70-90% breast cancer cases and have an important role in progression of breast cancer[8-15]. However intracellular relationship with androgen in breast cancer is not well understood. Here in present study, we report that androgen (DHT) via p21, inhibits TNF-á induced JNK1 activation, thereby suppressing apoptosis**.** Androgen inhibits TNF-á induced JNK activation and cell death via AR-mediated up-regulation of p21.

Our results show that the inhibitory effect by androgen was abrogated by treatment with specific AR antagonist bicalutamide (Cdx), which blocks AR transcription activity, or the protein synthesis inhibitor CHX (cyclohexamide). Thus, the inhibition by androgen depends on AR-mediated transcription and de novo protein synthesis. Furthermore, our results show that p21, which is one of the androgen target genes [32], mediates the inhibition by androgen on TNF-á induced JNK activation and apoptosis. Previously, it has been reported that p21 was able to inhibit JNK itself directly [35] or inhibited mTOR-stimulated ASK1, which is one of the MAP3Ks for JNK [36].

We found that androgen not only inhibited JNK activity, but it also inhibited activation of JNK. Phosphorylation of JNK at Thr183 and Tyr185,

which are required for JNK activation by upstream kinases [31, 32], was also inhibited by androgen. These data suggest that androgen-induced p21 may inhibit upstream activators of JNK. Previous literature suggests that p21 inhibits TNF-á stimulated JNK by inhibiting ASK1. Future studies are needed to determine which JNK upstream activator in TNF-á signalling are inhibited by androgen-induced p21. Our findings that androgen via p21 inhibits JNK activation and thereby suppresses apoptosis induced by TNF-á may have important clinical implications. It has been hypothesized that androgen ablation, which causes regression of androgen-dependent prostate cancer, may inadvertently select those cancerous cells that can grow independently of androgen through various mechanisms [1]. AR seems to be predictor for response to hormonal therapy. Castellano I et.al 2010, had reported 30% reduction in mortality in ER positive breast cancer is associated with AR positivity [37].

Androgen receptor act as anti-proliferative effecter in ER positive breast cancer by antagonising ER, potentially inhibited the Estradiol mediated stimulation and trans-activation activity of ER [6], while in ER negative BCs AR positive facilitates the tumor growth in AR dependent manner and have intact and active AR signalling[38]. Androgen receptor positivity in ER negative breast cancer is associated with better disease free survival and low tumor burden and good prognosis as compared with AR negative breast cancer cases, while in ER positive breast cancer AR positivity is associated with very low lymph node involvement and androgen receptor is a independent prognostic marker for ER positive breast cancer [37, 39, 40]. High level of androgen receptor in postmenopausal women associated with increased risk of breast cancer [39].

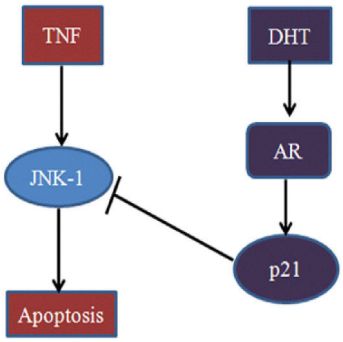

FIGURE 8: Summary: Androgen (DHT) inhibits the TNF-á induced JNK activation and apoptosis in p21 dependent manner.

CONCLUSION

DHT inhibits the TNF-á induce JNK activation and apoptosis in p21 dependent manner in breast cancer (MCF-7) cells.

REFERENCES

1. Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. Nature reviews Cancer. 2001;1(1):34-45. Epub 2002/03/20. doi: 10.1038/35094009. PubMed PMID: 11900250.
2. Anderson KM, Liao S. Selective retention of dihydrotestosterone by prostatic nuclei. Nature. 1968;219(5151):277-9. Epub 1968/07/20. doi: 10.1038/219277a0. PubMed PMID: 5671431.
3. Kokontis JM, Hay N, Liao S. Progression of LNCaP prostate tumor cells during androgen deprivation: hormone-independent growth, repression of proliferation by androgen, and role for p27Kip1 in androgen-induced cell cycle arrest. Molecular endocrinology (Baltimore, Md). 1998;12(7):941-53. Epub 1998/07/11. doi: 10.1210/mend.12.7.0136. PubMed PMID: 9658399.
4. Yeh S, Hu YC, Wang PH, Xie C, Xu Q, Tsai MY, et al. Abnormal mammary gland development and growth retardation in female mice and MCF7 breast cancer cells lacking androgen receptor. The Journal of experimental medicine. 2003;198(12):1899-908. Epub 2003/12/17. doi: 10.1084/jem.20031233. PubMed PMID: 14676301; PubMed Central PMCID: Pmc2194158.
5. Li S, Han B, Liu G, Li S, Ouellet J, Labrie F, et al. Immunocytochemical localization of sex steroid hormone receptors in normal human mammary gland. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society. 2010;58(6):509-15. Epub 2009/12/23. doi: 10.1369/jhc.2009.954644. PubMed PMID: 20026671; PubMed Central PMCID: Pmc2874183.
6. Peters AA, Buchanan G, Ricciardelli C, Bianco-Miotto T, Centenera MM, Harris JM, et al. Androgen receptor inhibits estrogen receptor-alpha activity and is prognostic in breast cancer. Cancer research. 2009;69(15):6131-40. Epub 2009/07/30. doi: 10.1158/0008-5472.can-09-0452. PubMed PMID: 19638585.
7. Zhou J, Ng S, Adesanya-Famuiya O, Anderson K, Bondy CA. Testosterone inhibits estrogen-induced mammary epithelial proliferation

and suppresses estrogen receptor expression. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2000;14(12):1725-30. Epub 2000/09/07. doi: 10.1096/fj.99-0863com. PubMed PMID: 10973921.

8. Hall RE, Aspinall JO, Horsfall DJ, Birrell SN, Bentel JM, Sutherland RL, et al. Expression of the androgen receptor and an androgen-responsive protein, apolipoprotein D, in human breast cancer. British journal of cancer. 1996;74(8):1175-80. Epub 1996/10/01. doi: 10.1038/bjc.1996.513. PubMed PMID: 8883401; PubMed Central PMCID: Pmc2075941.

9. Kuenen-Boumeester V, Van der Kwast TH, Claassen CC, Look MP, Liem GS, Klijn JG, et al. The clinical significance of androgen receptors in breast cancer and their relation to histological and cell biological parameters. European journal of cancer (Oxford, England : 1990). 1996;32a(9):1560-5. Epub 1996/08/01. doi: 10.1016/0959-8049(96)00112-8. PubMed PMID: 8911118.

10. Isola JJ. Immunohistochemical demonstration of androgen receptor in breast cancer and its relationship to other prognostic factors. The Journal of pathology. 1993;170(1):31-5. Epub 1993/05/01. doi: 10.1002/path.1711700106. PubMed PMID: 8100853.

11. Park S, Koo J, Park HS, Kim JH, Choi SY, Lee JH, et al. Expression of androgen receptors in primary breast cancer. Annals of oncology : official journal of the European Society for Medical Oncology. 2010;21(3):488-92. Epub 2009/11/06. doi: 10.1093/annonc/mdp510. PubMed PMID: 19887463.

12. Søreide JA, Lea OA, Varhaug JE, Skarstein A, Kvinnsland S. Androgen receptors in operable breast cancer: relation to other steroid hormone receptors, correlations to prognostic factors and predictive value for effect of adjuvant tamoxifen treatment. European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology. 1992;18(2):112-8. Epub 1992/04/01. PubMed PMID: 1582503.

13. Suzuki T, Miki Y, Takagi K, Hirakawa H, Moriya T, Ohuchi N, et al. Androgens in human breast carcinoma. Medical molecular morphology. 2010;43(2):75-81. Epub 2010/08/05. doi: 10.1007/s00795-010-0494-3. PubMed PMID: 20683693.

14. Gucalp A, Traina TA. Triple-negative breast cancer: role of the androgen receptor. Cancer journal (Sudbury, Mass). 2010;16(1):62-5. Epub 2010/02/19. doi: 10.1097/PPO.0b013e3181ce4ae1. PubMed PMID: 20164692.

15. Nahleh Z. Androgen receptor as a target for the treatment of hormone receptor-negative breast cancer: an unchartered territory. Future oncology (London, England). 2008;4(1):15-21. Epub 2008/02/05. doi: 10.2217/14796694.4.1.15. PubMed PMID: 18240997.

16. Tang F, Kokontis J, Lin Y, Liao S, Lin A, Xiang J. Androgen via p21 inhibits tumor necrosis factor alpha-induced JNK activation and apoptosis. The Journal of biological chemistry. 2009;284(47):32353-8. Epub 2009/09/03. doi: 10.1074/jbc.M109.042994. PubMed PMID: 19723627; PubMed Central PMCID: Pmc2781650.

17. Baud V, Karin M. Signal transduction by tumor necrosis factor and its relatives. Trends in cell biology. 2001;11(9):372-7. Epub 2001/08/22. doi: 10.1016/s0962-8924(01)02064-5. PubMed PMID: 11514191.

18. Locksley RM, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. Cell. 2001;104(4):487-501. Epub 2001/03/10. doi: 10.1016/s0092-8674(01)00237-9. PubMed PMID: 11239407.

19. Tracey KJ, Cerami A. Tumor necrosis factor: an updated review of its biology. Critical care medicine. 1993;21(10 Suppl):S415-22. Epub 1993/10/01. PubMed PMID: 8403979.

20. Hibi M, Lin A, Smeal T, Minden A, Karin M. Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. Genes & development. 1993;7(11):2135-48. Epub 1993/11/01. doi: 10.1101/gad.7.11.2135. PubMed PMID: 8224842.

21. Dai T, Rubie E, Franklin CC, Kraft A, Gillespie DA, Avruch J, et al. Stress-activated protein kinases bind directly to the delta domain of c-Jun in resting cells: implications for repression of c-Jun function. Oncogene. 1995;10(5):849-55. Epub 1995/03/02. PubMed PMID: 7898927.

22. Dérijard B, Hibi M, Wu IH, Barrett T, Su B, Deng T, et al. JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and

phosphorylates the c-Jun activation domain. Cell. 1994;76(6):1025-37. Epub 1994/03/25. doi: 10.1016/0092-8674(94)90380-8. PubMed PMID: 8137421.
23. Lin A. Activation of the JNK signaling pathway: breaking the brake on apoptosis. BioEssays : news and reviews in molecular, cellular and developmental biology. 2003;25(1):17-24. Epub 2003/01/01. doi: 10.1002/bies.10204. PubMed PMID: 12508278.
24. Chang L, Karin M. Mammalian MAP kinase signalling cascades. Nature. 2001;410(6824):37-40. Epub 2001/03/10. doi: 10.1038/35065000. PubMed PMID: 11242034.
25. Davis RJ. Signal transduction by the JNK group of MAP kinases. Cell. 2000;103(2):239-52. Epub 2000/11/01. doi: 10.1016/s0092-8674(00)00116-1. PubMed PMID: 11057897.
26. Karin M. The regulation of AP-1 activity by mitogen-activated protein kinases. The Journal of biological chemistry. 1995;270(28):16483-6. Epub 1995/07/14. doi: 10.1074/jbc.270.28.16483. PubMed PMID: 7622446.
27. Yu C, Minemoto Y, Zhang J, Liu J, Tang F, Bui TN, et al. JNK suppresses apoptosis via phosphorylation of the proapoptotic Bcl-2 family protein BAD. Molecular cell. 2004;13(3):329-40. Epub 2004/02/18. doi: 10.1016/s1097-2765(04)00028-0. PubMed PMID: 14967141.
28. Tang F, Tang G, Xiang J, Dai Q, Rosner MR, Lin A. The absence of NF-kappaB-mediated inhibition of c-Jun N-terminal kinase activation contributes to tumor necrosis factor alpha-induced apoptosis. Molecular and cellular biology. 2002;22(24):8571-9. Epub 2002/11/26. doi: 10.1128/mcb.22.24.8571-8579.2002. PubMed PMID: 12446776; PubMed Central PMCID: Pmc139858.
29. Kokontis JM, Liao S. Molecular action of androgen in the normal and neoplastic prostate. Vitamins and hormones. 1999;55:219-307. Epub 1999/02/09. doi: 10.1016/s0083-6729(08)60937-1. PubMed PMID: 9949683.
30. Karin M, Lin A. NF-kappaB at the crossroads of life and death. Nature immunology. 2002;3(3):221-7. Epub 2002/03/05. doi: 10.1038/ni0302-221. PubMed PMID: 11875461.
31. Lin A, Minden A, Martinetto H, Claret FX, Lange-Carter C, Mercurio F, et al. Identification of a dual specificity kinase that activates the Jun kinases and p38-Mpk2. Science (New York, NY). 1995;268(5208):286-90. Epub 1995/04/14. doi: 10.1126/science.7716521. PubMed PMID: 7716521.
32. Lu X, Nemoto S, Lin A. Identification of c-Jun NH2-terminal protein kinase (JNK)-activating kinase 2 as an activator of JNK but not p38. The Journal of biological chemistry. 1997;272(40):24751-4. Epub 1997/10/06. doi: 10.1074/jbc.272.40.24751. PubMed PMID: 9312068.
33. Waller AS, Sharrard RM, Berthon P, Maitland NJ. Androgen receptor localisation and turnover in human prostate epithelium treated with the antiandrogen, casodex. Journal of molecular endocrinology. 2000;24(3):339-51. Epub 2000/06/01. doi: 10.1677/jme.0.0240339. PubMed PMID: 10828827.
34. Lu S, Liu M, Epner DE, Tsai SY, Tsai MJ. Androgen regulation of the cyclin-dependent kinase inhibitor p21 gene through an androgen response element in the proximal promoter. Molecular endocrinology (Baltimore, Md). 1999;13(3):376-84. Epub 1999/03/17. doi: 10.1210/mend.13.3.0254. PubMed PMID: 10076995.
35. Shim J, Lee H, Park J, Kim H, Choi EJ. A non-enzymatic p21 protein inhibitor of stress-activated protein kinases. Nature. 1996;381(6585):804-6. Epub 1996/06/27. doi: 10.1038/381804a0. PubMed PMID: 8657286.
36. Huang S, Shu L, Dilling MB, Easton J, Harwood FC, Ichijo H, et al. Sustained activation of the JNK cascade and rapamycin-induced apoptosis are suppressed by p53/p21(Cip1). Molecular cell. 2003;11(6):1491-501. Epub 2003/06/25. doi: 10.1016/s1097-2765(03)00180-1. PubMed PMID: 12820963.
37. Castellano I, Allia E, Accortanzo V, Vandone AM, Chiusa L, Arisio R, et al. Androgen receptor expression is a significant prognostic factor in estrogen receptor positive breast cancers. Breast cancer research and treatment. 2010;124(3):607-17. Epub 2010/02/04. doi: 10.1007/s10549-010-0761-y. PubMed PMID: 20127405.
38. Doane AS, Danso M, Lal P, Donaton M, Zhang L, Hudis C, et al. An estrogen receptor-negative breast cancer subset characterized by a hormonally regulated transcriptional program and response to androgen. Oncogene. 2006;25(28):3994-

4008. Epub 2006/02/24. doi: 10.1038/sj.onc.1209415. PubMed PMID: 16491124.
39. Agoff SN, Swanson PE, Linden H, Hawes SE, Lawton TJ. Androgen receptor expression in estrogen receptor-negative breast cancer. Immunohistochemical, clinical, and prognostic associations. American journal of clinical pathology. 2003;120(5):725-31. Epub 2003/11/12. doi: 10.1309/42f0-0d0d-jd0j-5edt. PubMed PMID: 14608899.
40. Schippinger W, Regitnig P, Dandachi N, Wernecke KD, Bauernhofer T, Samonigg H, et al. Evaluation of the prognostic significance of androgen receptor expression in metastatic breast cancer. Virchows Archiv : an international journal of pathology. 2006;449(1):24-30. Epub 2006/04/22. doi: 10.1007/s00428-006-0213-6. PubMed PMID: 16628414.