

Review

Molecular roles of MAP kinases and FADD phosphorylation in prostate cancer

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Summary. Mitogen activated protein (MAP) kinases are well known serine threonine kinases that modulate gene expression, mitosis, cell proliferation and programmed cell death or 'apoptosis' in response to various stresses. Extracellular stress regulated kinase (ERK), c-jun NH2 terminal kinase and p38 are major members of the MAP kinases, and there is now a body of evidence of their involvement in genesis or sensitivity to chemotherapy of human prostate cancers. In this review, we focus on the molecular roles of MAP kinases and their pathological correlations, with particular attention to novel downstream signals through phosphorylation of the Fas-associated death domain protein that effectively regulates not only apoptosis but also the cell cycle in prostate neoplastic cells.

Key words: MAP kinase, Apoptosis, FADD phosphorylation, Prostate cancer

Introduction

Prostate cancer is a common malignancy in American and to a lesser extent Japanese men. Improved procedures for surgical intervention and radiation have significantly reduced the number of fatalities but this type of cancer often becomes highly resistant after chemotherapy, and there is still no effective cure for patients with advanced disease, especially in hormone-independent cases. One possible therapeutic approach is to induce apoptosis in prostate cancer and for this purpose, mitogen activated protein (MAP) kinases are main targets, known to be involved in both anti- and pro-apoptotic pathways. There are three major members of the MAP kinase family: extracellular stress regulated kinase (ERK) (isoforms are ERK1 and 2), c-jun NH2 terminal kinase (JNK) (isoforms are JNK1, 2 and 3) and p38 (isoforms are p38 MAP kinases α , β , γ and δ) (Davis, 2000). It is widely accepted that activation of

ERK contributes to cell differentiation, proliferation and survival (Xia et al., 1995); in contrast, JNK and p38, which are activated by pro-inflammatory cytokines and environmental stresses, promote apoptosis (Wilkinson et al., 1998). However, pathological correlations and the downstream targets of these kinases remain unclear. One problem is that these may vary with the extracellular stimulation, therapeutic context and individual patient characteristics (Maroni et al., 2004). We provide here an update of published studies examining molecular and pathological aspects of MAP kinases, especially ERK, JNK and p38, in prostate cancer tissues or cell lines.

MAP kinase signaling

As shown in Fig. 1, ERK, JNK and p38 are activated by the upstream MAP kinase kinases (MKK), and in turn, by more upstream kinases, including Raf and the MAP/ERK kinase (MEK) kinases. Other MKK kinases include germinal center kinase (GCK), p21-activated kinase (PAK), TGF- β -activated kinase (TAK), and apoptosis signal regulating kinase 1 (ASK1) (Bagrodia et al., 1995; Pombo et al., 1995; Brown et al., 1996; Ichijo et al., 1997). In response to various stimuli such as heat shock and exposure to death receptor ligands like tumor necrosis factor α , Fas etc, irradiation, ultraviolet, osmotic pressure, or oxidative stress, MAP kinases transduce signals from the cell membrane to the nucleus and thereby contribute to a wide spectrum of cellular processes including growth, differentiation and apoptosis (Wada et al., 2004).

ERK activation in androgen receptor (AR) and the co-activators-mediated signals

Androgens and androgen receptors (ARs) are required for progression of human prostate cancer (Denmeade et al., 1996), and MAP kinase activation, especially of ERK, is one of a number of the AR signals that affect cell survival or death; Gioeli et al. (1999) have demonstrated that ERK activation is correlated with increasing Gleason score, whereas other investigators have suggested associations between

decline in ERK activity and advanced malignancy (Pawelczak et al., 2001; Malik et al., 2002). With regard to this possible inverse relationship of ERK with neoplasia, Uzgar et al. (2003) recently demonstrated with a TRAMP animal model that ERK activation is linked with prostatic epithelial proliferation and initiation of prostate cancer development, while ERK inactivation is correlated with the emergence of a poorly differentiated metastatic and androgen-independent phenotype. In addition, activated ERK has been shown to mediate activation of the androgen receptor and/or PSA secretion through the growth factor receptor tyrosine kinase, Her2/Neu, also known as erbB2, in androgen-independent prostate cancer cells (Fig. 2) (Grossmann et al., 2001).

AR itself exists as a phosphoprotein that can be transactivated through the phosphorylation signal cascade. The steroid receptor co-activator-1 (SRC-1) is the first identified member of the co-activator family that regulates steroid receptors including the AR, and enhances ligand -dependent transactivation to increase transcription of androgen-regulated genes (Bevan et al., 1999; Fukazawa et al., 1999). ERK activation has been found to be closely associated with co-activator functions and recently, Ueda et al. (2002) demonstrated that SRC-1 could be phosphorylated by ERK, which is required for the ligand-independent activation of ARs by IL-6 in the LNCaP prostate cancer cell line. On the other hand, the kinase Src is located upstream of ERK, and Src-MEK1/2-ERK1/2-cyclic AMP response element binding protein signaling mainly contributes to androgen

independence (Unni et al., 2004). Thus, AR co-activators may contribute to both androgen-dependent and independent development of prostate cancer via MAPK activation.

Growth factor signaling and ERK

The mitogenic effects of growth factors are of great significance for prostate cancer development (Ueda et al., 2002). EGF and insulin-like growth factor (IGF)-1 being potent mitogens produced and generated by stromal prostate tissue. EGF and IGF-1 also activate the AR independent of androgen stimulation as described in the previous section, suggesting that IGF-1 can act as a promoter of cancer metastasis (Ritchie et al., 1997). There is in fact some clinical evidence to support the participation of IGF-1 and EGF in prostate carcinogenesis, high serum levels of IGF-1 being significantly associated with an increased risk for prostate cancer (Chang et al., 1998). IGF-1 and EGF stimulate intracellular signaling pathways converging at the level of ERK2 (Putz et al., 1999), which is a key kinase mediator of growth factor-induced mitogenesis in prostate cancer cells (de Souza et al., 1997). The two major substrates of the IGF-1 receptor, insulin receptor substrate-1 (Chang et al., 1998) and Shc, are known to contribute to IGF-1-induced activation of ERK (Dews et al., 2000), and expression of an inactive mutant form of MEKK1 has been shown to cancel EGF-stimulated ERK activation (Fanger et al., 1997). Recent reports have pointed to 'cross talk' between EGF and IGF-1 receptors in mediating responses to IGF-1. IGF-1-mediated transactivation of the EGF receptor through the IRS-1/phosphatidylinositol-3-kinase/Akt pathway appears largely responsible for IGF-1-stimulated Shc phosphorylation and subsequent activation of the ERK

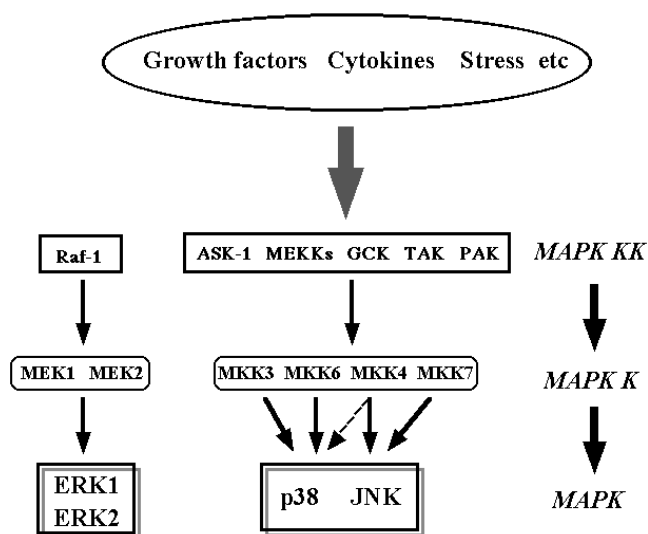


Fig. 1. Mitogen activated protein kinase (MAPK) signaling through the three major groups: ERK, p38, and JNK. These MAP kinases are activated by upstream kinases such as MAP kinase kinase (MAPKK) and the MAP/ERK kinases (MEKs) 1, 2, 3, 4, 6 or 7, which, in turn, are activated by several different MAP kinase kinase kinases (MAPKKKs) such as ASK-1, MEKK, GCK, TAK or PAK. Numerous stimulatory elements, including growth factors, can activate MAPKKKs.

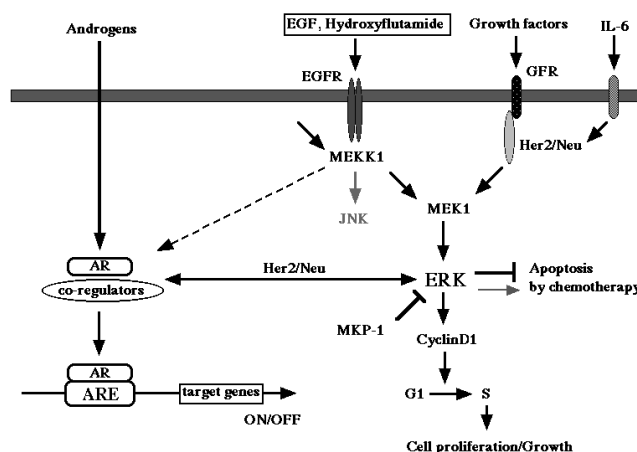


Fig. 2. Androgen receptor activation in prostate cancer cells. The androgen receptor can be activated not only by androgen receptor agonists, but also non-steroid factors such as growth factors and cytokines. Within these activation pathways, MAP kinases and ERK, in particular, play very important roles.

cascade (Roudabush et al., 2000).

Expression of activated ERK in prostate cancer

The status of activated or phosphorylated ERK in human prostate cancer cells has been shown to correlate with pathological parameters and expression of phospho-MAPK increases in line with the Gleason score, suggesting activation of ERK signaling with prostate cancer progression (Gioeli et al., 1999). High expression of phosphorylated ERK has been detected in androgen independent prostate cancer specimens (Lee et al., 2002), probably due to androgen antagonist-mediated EGF receptor activation independent of the ligand. However, conflicting reports have accumulated as to the pathological significance of ERK activation, including decreased expression of phospho-ERK with progression of prostate cancer (Paweletz et al., 2001; Malik et al., 2002).

p38 activation and cell survival

p38 responds to various stimuli, including UV radiation, oxidative stress, anticancer drugs, heat shock, osmotic shock, and inflammatory cytokines (Dong et al., 2002) and its effects on prostate cancer cells are complex with dual cytotoxic and cytoprotective functions. We previously demonstrated that p38 is necessary for apoptosis induced by an endogenous metabolite of estradiol-17 β ,2-methoxyestradiol (2-ME) in human prostate cancer cells (Shimada et al., 2003). It was thus established that p38 combined with JNK stabilizes p53 through NF kappaB/AP-1 signals, which are essential for apoptosis induction. Very recently, evidence was presented of an importance of p38 for Smad7-mediated apoptotic pathway induced by 2-ME (Davoodpour et al., 2005). In addition, p38 plays an important role in induction of apoptosis by grape seed extract (Vayalil et al., 2004) or phorbol 12-myristate 13-acetate/protein kinase C (Tanaka et al., 2003) in prostate cancer cells. Thus p38 may contribute to suppression of prostate cancer development. At the same time, this kinase can directly affect invasiveness of prostate cancer, mediated by the G protein-coupled P2Y receptor (Chen et al., 2004). Moreover, an essential role for p38 in hypoxia-induced tumor cell invasion has been indicated through upregulation of urokinase plasminogen activator receptors (Lee et al., 2004). Crosstalk between the p38 kinase and Smad signaling pathways appears essential for TGF- β -mediated cell adhesion and interleukin-6 expression in prostate cancer cells (Hayes et al., 2003; Park et al., 2003).

The picture is further complicated by reports suggesting that p38 suppresses cell proliferation by inhibiting ERK (Aguirre-Ghiso et al., 2001) inducing G0-G1 arrest (Molnar et al., 1997) or triggering senescence (Haq et al., 2002). Recently the level of active phospho-ERK coupled to the ERK: p38 activity ratio has been shown to be predictive of in vivo behavior

in ~90% of cancer cell lines tested, including examples derived from prostate cancers (Aguirre-Ghiso et al., 2001). Thus, a low ERK: p38 ratio favors tumor growth, while a high value predisposes to growth arrest. Clearly, interactions between MAP kinases, as well as any effects on single elements need to be considered in assessing the full kinase contribution to prostate carcinogenesis.

JNK activation and cell survival

It is well known that JNK is activated and contributes to induction of apoptosis with a variety of extracellular stresses (Ip et al., 1998), but its impact on prostate cancer cell survival remains controversial. Activated JNK can phosphorylate the jun transcription proteins c-jun, junB, and junD, and therefore control cell life or death. Interleukin-6 is induced by TGF- β 1 through multiple mechanisms, including the JNK-c-jun pathway, which seems to contribute to the oncogenic switch in prostate carcinogenesis, in part by counteracting the growth suppressive function of TGF- β 1 (Park et al., 2003; Yang et al., 2003). It is generally understood that c-jun enhances cell proliferation through

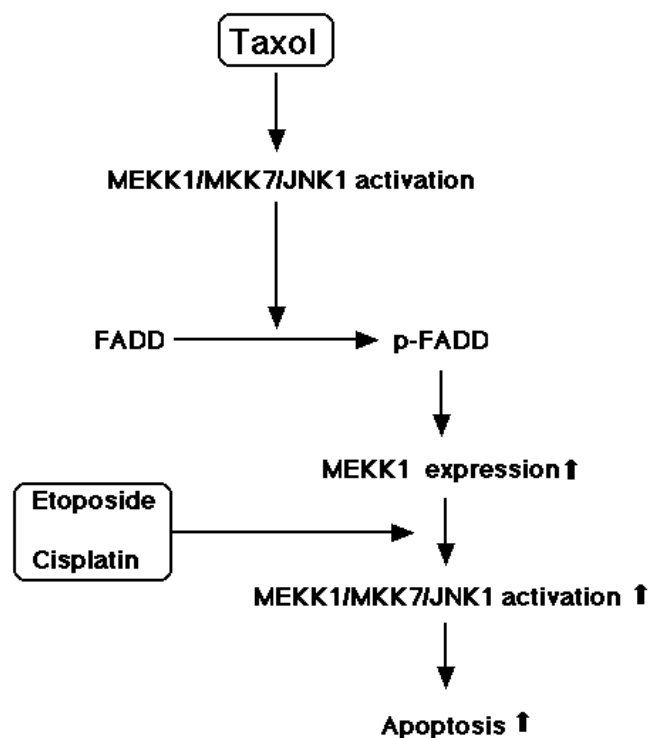


Fig. 3. A novel chemotherapy-induced apoptotic signaling cascade in prostate cancer cells associated with JNK and FADD phosphorylation. The MEKK1/MKK7/JNK1 pathway mediates FADD phosphorylation at 194 serine; phosphorylated FADD can subsequently upregulate MEKK1 and downstream JNK1 activation which play essential roles in sensitization to apoptosis induced by anticancer drugs (from data using etoposide or cisplatin combined with paclitaxel (Shimada et al., 2004)).

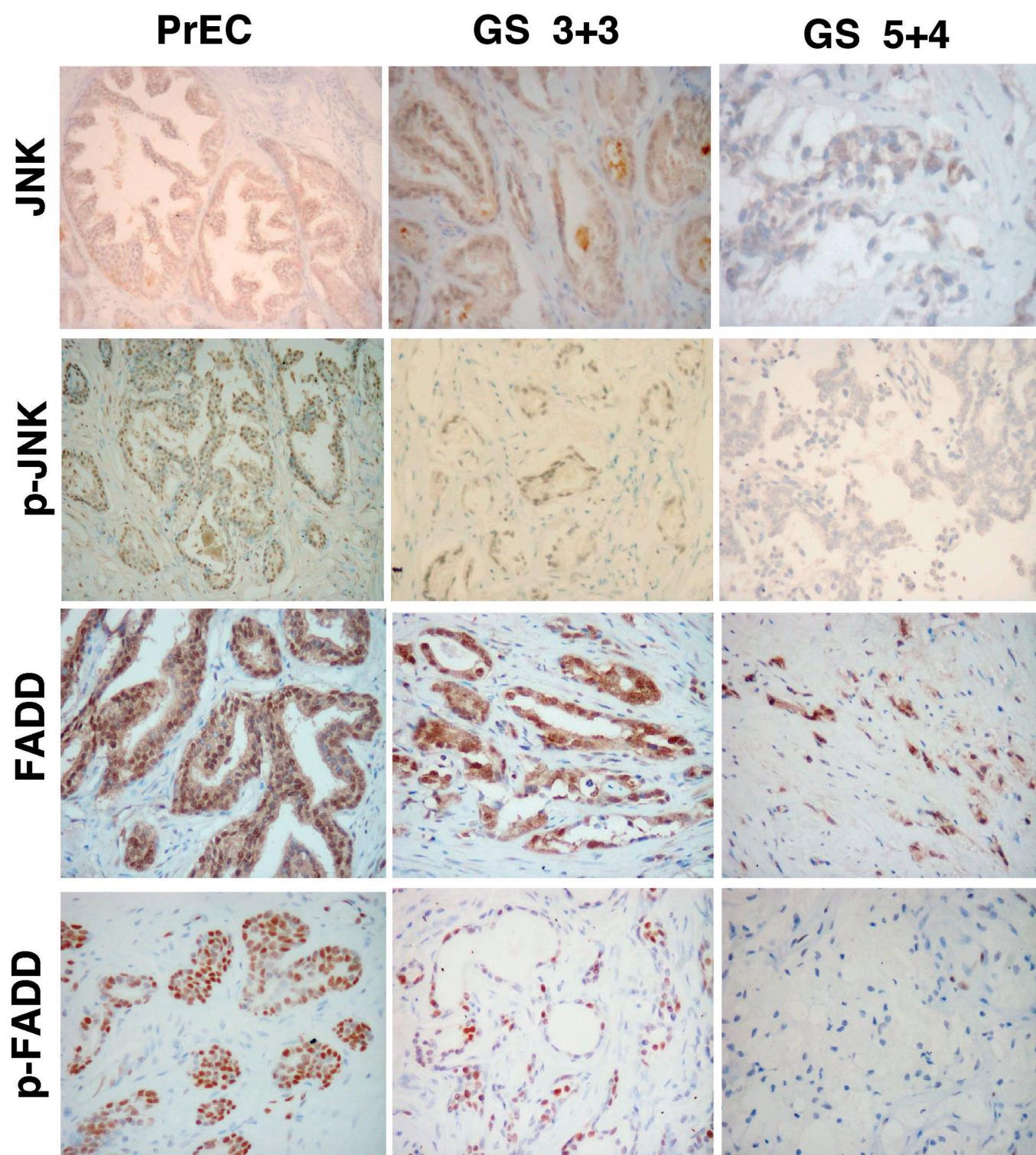


Fig. 4. Phosphorylation of JNK and FADD in normal prostate epithelial cells and cancer cells. Normal prostate epithelial cells ((A)-1,2 and (B)-1,2) and cancer cells which were obtained from Gleason score 6 (3+3) and 9 (5+4) tumors ((A)-3 to 6 and (B)-3 to 6), were stained with anti-JNK, anti-FADD, antiphosphorylated JNK or anti-phosphorylated FADD antibodies. Original magnification: x 100-200

inhibition of tumor suppressor gene expression and induction of cyclin D1 transcription, both of which are antagonized by junB (Grosch et al., 2003). This c-jun/junB antagonism might explain the biphasic effects of JNK on tumor progression, even though it has yet to be determined whether or not jun proteins actually function in prostate cancer cells.

We here focus on two major isoforms, JNK1 and 2, in the view of their disparate roles. JNK1 is a crucial suppressor of tumor development in the skin, whereas JNK2 promotes tumor formation (She et al., 2002). Potapova et al. (2000) found that JNK2 inhibition leads to growth suppression and apoptosis induction in a p53 dependent manner using several human tumor cell lines.

p38/JNK expression or activation in human prostate tissues

p38 is expressed in non-neoplastic prostate epithelial or basal cells, but not activated (Hemmi et al., 1998), while being strongly activated in hyperplastic or cancer cells. In a transgenic mouse model for prostate cancer, p38 has been demonstrated to be expressed through all stages of tumorigenesis from prostatic intraepithelial neoplasia (PIN) to well-differentiated, and moderately differentiated cancers (Uzgare et al., 2003), but without significant activation. In contrast, both p38 and JNK are reduced or absent in poorly differentiated cancers or metastatic lesions.

Prostate progenitor cells and MAP kinases

Survival signals in stem cells and derived progenitor cells, so-called transit amplifying cells, are a subject of keenest interest for their potential to provide a rare explanation for prostatic carcinogenesis. Progenitor cells are a subset of proliferating epithelial cells within the prostatic glands whose transformed progeny appear to give rise to prostate cancer. Overactivation of the key signals involved in proliferation or growth of the progenitor cells might trigger prostate cancer, including the Hedgehog signals which are essential for progenitor cell growth and prostate tissue renewal following castration-induced androgen withdrawal, and for tumors from progenitor-like cells transfected with the target gene, Gli, in athymic mice (Karhadkar et al., 2004). In addition, prostate progenitor cells have been recently shown to require MAP kinases, especially ERK and JNK, for survival (Uzgare et al., 2004). Taken together, all types of MAP kinases are necessary for early processes in prostate carcinogenesis.

FADD phosphorylation: a novel down- and upstream MAP kinase target

(1) JNK/FADD phosphorylation contributes to chemosensitivity

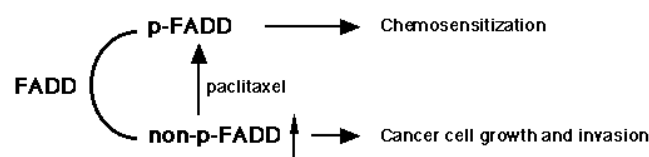
FADD is phosphorylated exclusively at the C-

terminal serine 194, specifically in the G2/M transition, suggesting an essential role in cell cycle progression (Scaffidi et al., 2000). We here clearly demonstrated that a G2 blocker, paclitaxel, arrests prostate cancer cells at G2/M and induces FADD phosphorylation at 194 serine dependent on JNK activation. Of interest, expression of upstream kinase of JNK, MEKK1, is increased by paclitaxel through phosphorylated FADD, and this up-regulation results in enhancement of anticancer drug-induced JNK activation and chemosensitization (Shimada et al., 2004). Our observations indicate that the close interaction of JNK activation with FADD phosphorylation is a key mechanism by which cell growth and/or proliferation are down-regulated in response to chemotherapeutic agents in prostate cancer cells (Fig. 3). Immunohistochemical studies have revealed much higher phosphorylation of JNK and FADD in normal epithelial cells than in cancers, although total expression of JNK and FADD was found to be similar in both cases (Shimada et al., 2005). Moreover, a statistically significant correlation between positivity for phosphorylated JNK and FADD was established (Fig. 4).

(2) The role of FADD phosphorylation in cancer cell growth and invasion

In normal epithelial cells, overexpression of a

Prostate cancer cells



Normal prostate epithelial cells

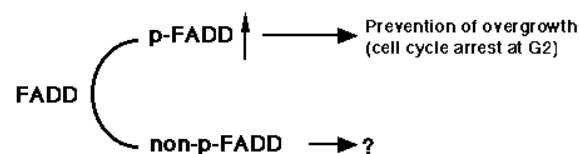


Fig. 5. Schematic presentation of the role of FADD phosphorylation in normal prostate epithelial cells and cancer cells. In normal cells, induction of phosphorylated FADD leads to cell cycle arrest at G2/M, controlling overgrowth. In prostate cancer cells, FADD is more dephosphorylated than in normal epithelial cells and acts to drive the cell cycle, enhancing colony formation and matrigel invasion *in vitro*. In addition, paclitaxel can induce FADD phosphorylation, resulting in enhancement of apoptosis due to anticancer drugs. Thus, the balance between phosphorylated and non-phosphorylated FADD at 194 may be closely associated with carcinogenesis and the development in human prostate cancer.

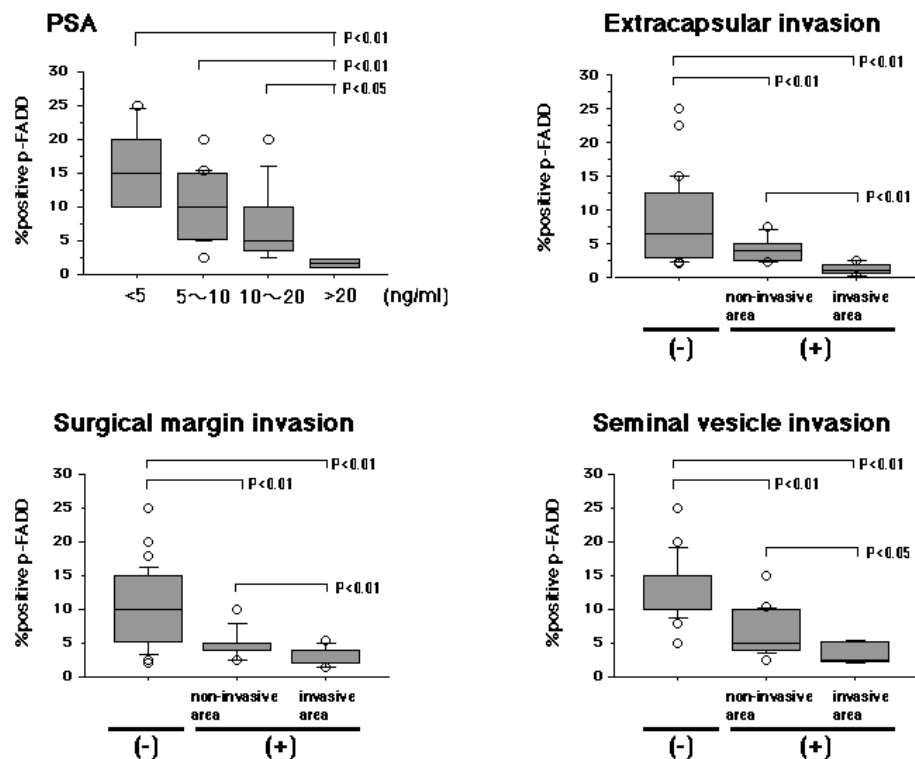


Fig. 6. Correlation between FADD phosphorylation and clinicopathological parameters. Box plots show positivity of phosphorylated FADD versus PSA and extracapsular, surgical margin and seminal vesicle invasion for cancer cells obtained from radical prostatectomy samples without neoadjuvant chemohormonal therapy. The Wilcoxon's rank sum test was used to analyze the distribution of percentages of phosphorylated FADD positive cells in relation to the morphology.

phosphorylation mimicking mutant FADD (S194E) causes G2/M cell cycle arrest, while a non-phosphorylation mimicking mutant (S194A) lacks this effect, overexpression resulting in cell cycle progression and enhanced colony formation and matrigel invasion (Shimada et al., 2005). Since S194E FADD overexpression alone did not significantly induce apoptosis in either normal epithelial or cancer cells, phosphorylation alone does not affect cell cycle and cell death, but rather appears to accelerate the apoptotic signaling initiated by anticancer drugs. Induction of phosphorylated FADD results not only in chemosensitization but also suppression of cancer cell growth and invasion through reduction in the non-phosphorylated form (Fig. 5). Moreover, we found the positivity for phosphorylated FADD in cancer cells to be significantly low in patients with a Gleason score ≥ 7 , serum PSA more than 20 ng/ml, a positive surgical margin or extracapsular or seminal vesicle invasion (Fig. 6). Of interest, expression of phosphorylated FADD is most prominent in cancer cells of the invasive area. The results indicate that transition from non-phosphorylated FADD to the phosphorylated form through JNK actions might be linked to successful chemotherapy. Moreover, FADD phosphorylation may be a useful new biomarker for prediction of cancer progression.

Conclusions

Despite significant advances in our understanding of the molecular roles of MAP kinases, no specific signals for cancer cell death or proliferation can be definitively assigned. Indeed, there are many conflicting reports regarding biological effects mediated by MAP kinases. As demonstrated in the present review, the influence of each specific enzyme, whether ERK, p38 or JNK varies with the stage in neoplastic development. Combination effects of MAP kinases should be borne in mind and data regarding inhibition or activation of a single kinase may not provide clinically appropriate information.

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