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Mitogen-activated protein kinases and apoptosis in PIN

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Abstract Mitogen-activated protein (MAP) kinases are key elements of the signalling systems needed to transduce different extracellular messages into cellular responses. At least three parallel MAP kinase pathways have been identified: one, stimulated by serum and growth factors to activate extracellular signal-regulated protein kinases (ERKs) by dual tyrosine and threonine phosphorylation, triggers cell proliferation or differentiation; the other two, induced by a variety of cellular stresses to activate c-jun N-terminal kinases (JNKs) and reactivating kinase (p38/RK), result in growth arrest and induction of apoptosis. Mitogen-activated protein kinase phosphatases (MKPs) inactivate MAP kinases through dephosphorylation and, thus, can modulate the MAP kinase pathways. Expression of JNK-1, ERK-1, p38/RK and MKP-1 proteins was investigated by immunohistochemistry and expression of MKP-1 mRNA by in situ hybridisation in 50 cases of high-grade prostatic intraepithelial neoplasia (PIN), thought to represent the precursor of prostate cancer. The frequency of apoptotic cells was also determined in these cases. Overexpression of the three MAP kinases and MKP-1 mRNA was found in all cases of high-grade PIN compared with normal prostate. Immunoreactivity for MKP-1 protein was found to be as intense as in normal glands in 30% and weaker in 56% of the PIN cases. Fourteen per cent of PIN cases did not stain with MKP-1 antibody. The proportion of apoptosis was significantly higher ($P < 0.008$) in PIN lesions that did not express MKP-1 protein than in those that did. These results are consistent with our previous demonstration of preferential inhibition of the apoptosis-related kinases by MKP-1 and further support the contention that MKP-1, even in PIN, may shift the balance existing between cell proliferation and death. When expressed, it may inhibiting those pathways that lead to apoptosis.

Key words Prostate · Mitogen-activated protein kinases · Apoptosis

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Introduction

Mitogen-activated protein (MAP) kinases are a family of serine/threonine kinases regulated by distinct extracellular stimuli. They represent key elements in highly conserved signalling pathways used by eukaryotic cells to transduce different extracellular messages into such diverse cellular responses as cell growth or cell death (apoptosis) [47]. Three MAP kinase groups have been extensively investigated in humans: extracellular signal-regulated protein kinases or ERKs, c-jun N-terminal kinases/stress-activated protein kinases or JNKs/SAPKs, and re-activating kinase or p38/RK [8, 17, 21]. There is substantial evidence indicating that activation of ERKs is a key event in cell signalling via *ras* and plays a central part in the induction of cell growth [23, 30, 31]. JNKs/SAPKs and p38/RK mediate signals in response to environmental stress and cytokines, transmit the effect of cellular insults or injury to the nucleus to influence gene expression and have an adverse influence on cell growth [26, 49].

These MAP kinases are activated by different stimuli. Growth factors, hormones, and mitogens preferentially activate ERKs [6], whereas inflammatory mediators (tumour necrosis factor-α or TNF-α, interleukin-1 or IL-1), UV light, and various forms of stress, including hormone withdrawal, preferentially activate JNKs/SAPKs and p38/RK [24], but do not cause significant phosphoryla-

tion of ERKs [11, 13, 41]. The pathway stimulated by serum and growth factors to activate ERKs triggers cell proliferation (with transient activation of ERKs) or differentiation (with sustained activation of ERKs) [9]. Another pathway, induced by a variety of cellular stresses to activate JNKs/SAPKs, leads to the activation of c-jun and results in growth arrest and stimulation of apoptosis [6, 20, 43, 49]. By a similar mechanism, extracellular stimuli such as UV light, osmotic shock, TNF-α, or IL-1 activate the p38/RK-related pathway [18]. p38/RK is the target of a series of anti-inflammatory agents termed CSAID (cytokine-suppressing anti-inflammatory drugs) that inhibit inflammatory cytokine secretion [26], and also of mediators of apoptosis [49]. Since a signal can be transmitted down parallel tracks, these phosphorylation cascades are activated to different extents, depending on the particular stimulus and the various other signalling components involved.

MAP kinases are regulated by reversible phosphorylation on tyrosine and threonine residues. Dual-specificity phosphatases, such as MKP-1, are proteins that dephosphorylate both phosphotyrosine and phosphothreonine residues. MKP-1, an immediate early gene whose expression is induced both by growth factors [7, 38, 42] and by stress [22, 27, 46], is either expressed or overexpressed in a variety of tissues and lesions [28]. In prostate cancer, it has been shown that MKP-1 can differentially regulate the interaction or cross-talk between the MAP kinase pathways and might be a key control point of their relative activities [29]. In particular, it has been suggested that the selective inhibition of the stress-activated cascades by MKP-1 may be essential for mitogenic signals to induce a productive response [16, 28]. The aim of the present study was to examine the expression of MAP kinases and MKP-1 in high-grade prostatic intraepithelial neoplasia, i.e., the direct precursor of prostate cancer [4, 5, 33], and to correlate their expression to the frequency of apoptosis in these lesions.

Materials and methods

Fifty cases of formalin-fixed paraffin-embedded tissue blocks were selected on the basis of the presence of high-grade PIN from radical prostatectomy cases from the files of the Beth Israel-Deaconess Medical Center, West Campus, Harvard Medical School and the Institute of Pathological Anatomy and Histopathology of the University of Ancona. Patients did not receive any hormonal treatment prior to surgery. Serial sections 5 μm thick were cut from each block and mounted on positively charged Super Frost Plus slides (Fisher Scientific, Santa Clarita, Calif.). One section was stained with haematoxylin and eosin (H&E), and examined by light microscopy to confirm the presence of prostatic intraepithelial neoplasia (PIN) on the block. The same section was used to count the number of apoptotic cells by light microscopy, utilizing established criteria [35]. Criteria for diagnosing PIN have already been described elsewhere[14]. 'PIN' is used here as a synonym for high-grade PIN. In addition, foci of normal prostate and prostatic adenocarcinoma in the slides containing PIN were studied. Ten cases of normal prostate from patients with bladder cancer were included as controls.

Immunohistochemistry was carried out in an automated Ventana 320/ES immunohistochemistry instrument, (Ventana Medical Systems, Tucson, Ariz.) [29]. Paraffin sections were deparaffinised and rehydrated. Endogenous peroxidase activity was quenched by incubating sections in 0.6% H_2O_2 in methanol for 15 min. To unmask antigens, slides were heated inside a pressure cooker, in a microwave oven at 700 W for 30 min in 0.01 M citrate buffer, pH 6.0 (Biogenex, San Ramon, Calif.). Positive controls (peripheral nerve and ganglion for MKP-1, ERK-1, and JNK-1 and polymorphonuclear leukocytes for p38/RK) were run simultaneously. For negative control, a mouse monoclonal antibody (MOPC-21') not directed against any known human epitope was used [40].

Sections were incubated with polyclonal antibodies against MKP-1 (dilution 1:50), ERK (dilution 1:20), JNK-1 (dilution 1:20) and p38/RK (dilution 1:100) (Santa Cruz Biotechnology, Santa Cruz, Calif.). The immune reaction was revealed by the instrument utilising the avidin-biotin complex (ABC) method with diaminobenzidine (DAB) as the chromogen, with standardised development times.

For in situ hybridisation (ISH), 1 μg of the recombinant Bluescript-SK plasmid (Stratagene, La Jolla, Calif.) containing a 200 base pair rat MKP-1 insert (encompassing the catalytic domain; homology to human: 97%) [32] was linearised by SalI and EcoRI and transcribed using T3 and T7 RNA polymerase in a mixture of ATP, CTP and GTP, as well as UTP and digoxigenin-UTP (6.5 and 3.5 mM respectively) (Boerhinger Mannheim, Indianapolis, Ind.), to generate sense and antisense RNAs, respectively [29].

In situ hybridisation with digoxigenin-labelled riboprobes was performed on an automated instrument (Gen II, Ventana Medical Systems, Tucson, Ariz.) in which duration and temperature of all the steps were standardised, as previously described [28, 29]. Briefly, sections were digested with proteinase K for 8 min at 37°C. The highest stringency of post-hybridisation washes was 52°C in 0.1xSSC for 15 min each. Alkaline phosphatase-conjugated anti-digoxigenin antibody (1:1000) was applied for 28 min at 37°C. Detection was accomplished with nitro blue tetrazolium/5-bromo 4-chloro 3-indolyl phosphate (NBT/BCIP) as a substrate for 8 min. Hybridisation controls were performed pretreating tissue sections with RNAse A for 1 h at 37°C and utilising sense MKP-1 probe. Preservation of the RNA was evaluated by hybridising the sections with a riboprobe for G3PDH.

The number of apoptotic cells per thousand (‰) was determined by counting, on each gland with high-grade PIN, the cell identified on the H&E section as apoptotic. A minimum of 2000 cells were evaluated for each case. Bonferroni/Dunn's multiple comparison procedure was used to analyse the statistical significance of the data. According to this method, comparisons are not significant unless the corresponding P-value is less than 0.017.

Results

The immunostaining of normal prostate from patients with bladder cancer was equivalent to that seen in the prostatectomy specimens. Immune reactivity for JNK-1 antibody was present in the cytoplasm of both secretory (+) and basal (+) cells; nuclear staining was seen in approximately 60% of the secretory cells (Fig. 1a). The polymorphonuclear leukocytes immunoreacted with JNK-1 antibody. ERK-1 protein was expressed in the cytoplasm of both secretory (+) and basal (+) cells; nuclear staining was observed in 5% of the basal cells; endothelial cells and some lymphocytes immunoreacted with the ERK-1 antibody (Fig. 1b). Cytoplasmic immunostaining for p38/RK protein was present in the basal (+) cells; in 10% of the cases examined, some degree (10% of the cells) of nuclear staining was seen in the same cells with p38/RK antibody (Fig. 1c). Fibromuscular tissue around the glands and the polymorphonuclear leukocytes also

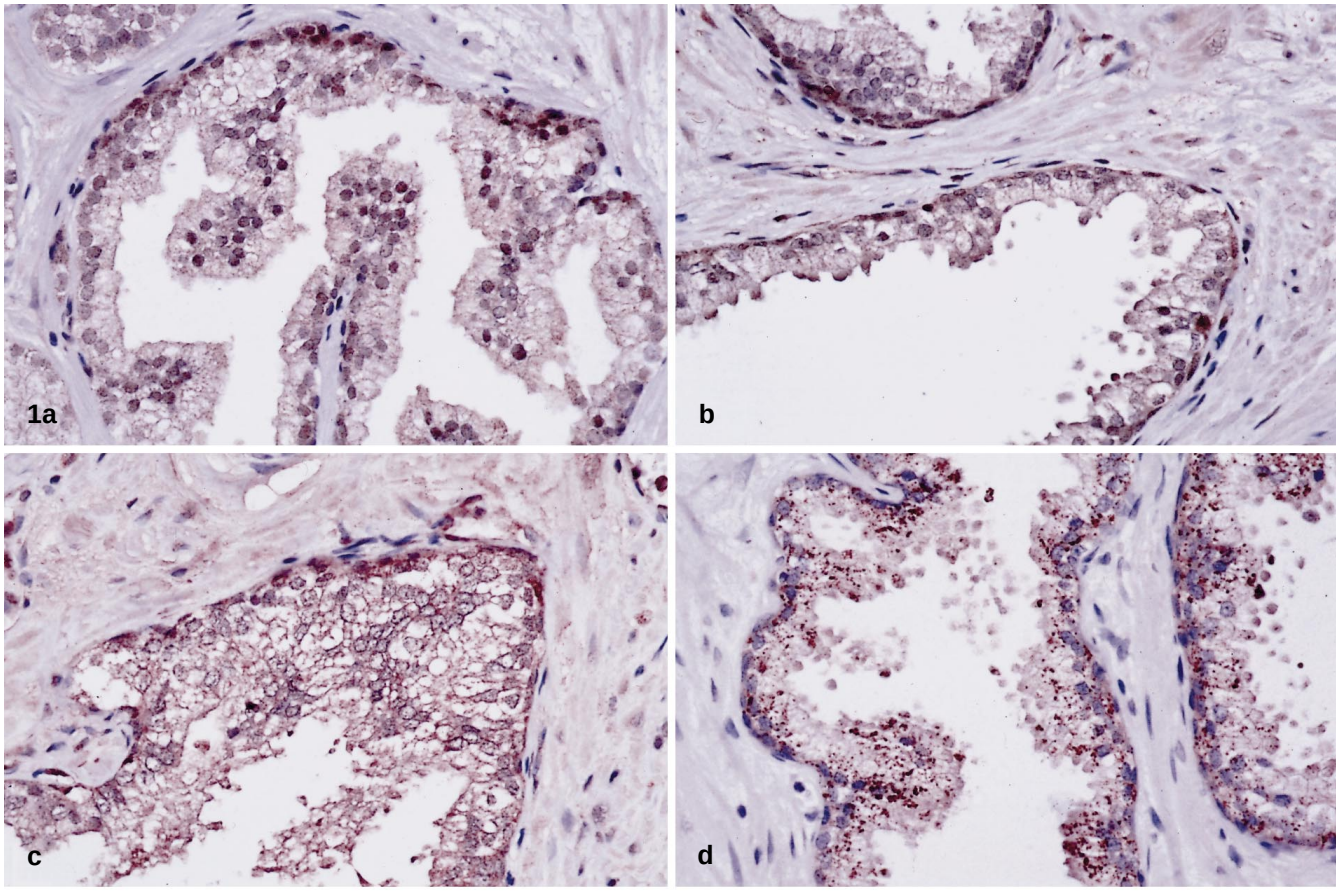

Fig. 1 Expression of **a** JNK-1, **b** ERK-1, **c** p38/RK and **d** MKP-1 proteins in normal prostatic glands. Original magnification × 200

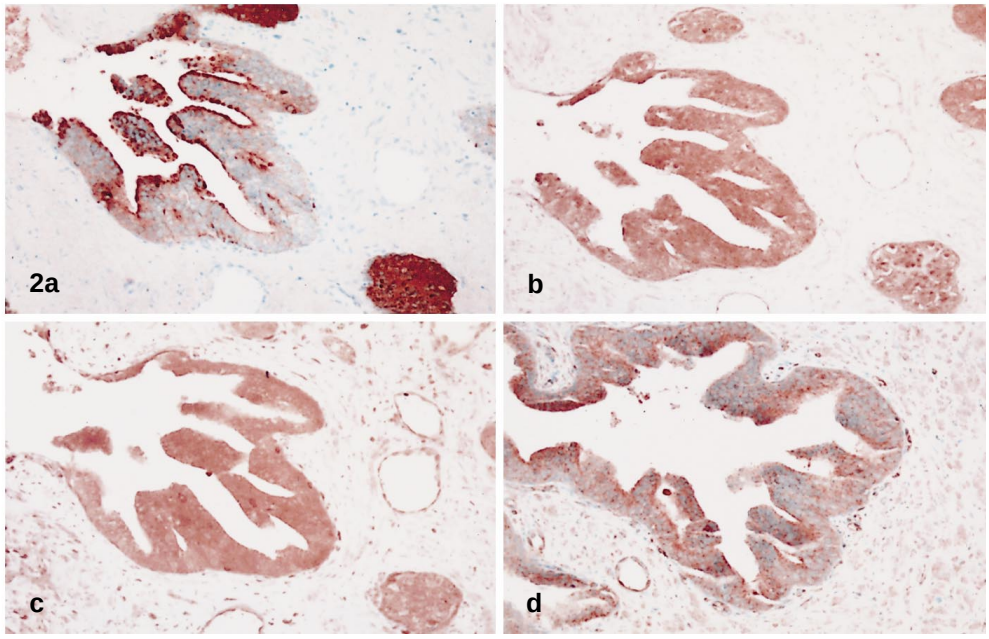

Fig. 2 Expression of **a** MKP-1, **b** JNK-1, **c** ERK-1 and **d** p38/RK proteins on serial sections of a high-grade prostatic intraepithelial neoplasia (PIN) case. Weak intensity of staining for MKP-1 protein is shown in **a**: note the condensation of the staining beneath the apical portion of the cell membrane. Original magnification × 200

reacted with this antibody. Secretory cells showed intense cytoplasmic expression (++) of MKP-1 protein; nuclear staining was occasionally present; basal cells staining was variable (±) (Fig. 1d). MKP-1 mRNA was expressed only in the cytoplasm of basal (+) cells (Table 1). MKP-1 protein positivity against the negativity for the corresponding mRNA has been reported before by our group [29]. This finding suggests a regulation of the transcriptional/translational processes, allowing the accumulation of proteins along with undetectable levels of the corresponding mRNA.

In high-grade prostatic intraepithelial neoplasia JNK-1, ERK-1, and p38/RK proteins were found to be uniformly intense (++), when compared to normal tissue, in the cyto-

Table 1 Expression of mitogen-activated protein kinases, MKP-1 protein and mRNA in normal prostate, high-grade (MAP) prostatic intraepithelial neoplasia (*PIN*) and prostatic adenocarcinoma (*AdenoCA*). Cytoplasmic staining intensity (+ normal tissue staining or weak staining referred to *PIN*, ++ intense or increased intensity of staining compared with normal tissue, ± variable staining, – negative

	JNK-1		ERK-1		p38/RK		MKP-1 protein		MKP-1 mRNA	
Normal prostate	Secretory Basal	+ +	Secretory Basal	+ +	Secretory Basal	– +	Secretory Basal	++ ±	Secretory Basal	– +
PIN	++		++		++		++ + –	30%[a] 56%[a] 14%[a]	++	
AdenoCA	++ +	80%[a] 20%[a]	++ +	80%[a] 20%[a]	++ +	80%[a] 20%[a]	++ + –	50%[a] 40%[a] 10%[a]	++ –	90%[a] 10%[a]

[a] Percentage of cases staining with different intensity; where the percentage is not shown this means that all cases were uniformly stained

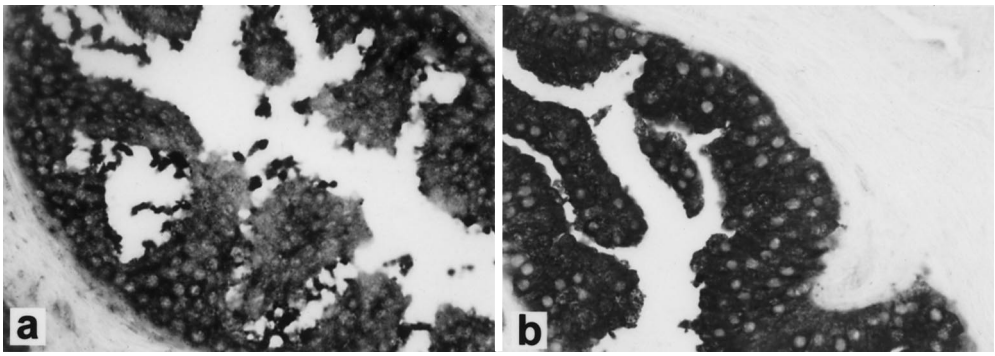

Fig. 3 **a** In situ expression of MKP-1 mRNA showing strong hybridisation signal in a high-grade PIN **b** displaying intense cytoplasmic expression of MKP-1 protein. Original magnification × 400

Table 2 Frequency of apoptosis in high-grade PIN in relation to intensity of staining for MKP-1 protein (++ intense, + weak, – negative, *SD* standard deviation)

MKP-1 protein (intensity of staining)	Apoptosis (frequency, ‰)
++	5.1±3.4 SD
+	9.2±3.5 SD
–	12.1±5.0 SD

plasm of cells in all high-grade PIN lesions examined (Fig. 2). Occasional nuclear staining was detected with the same antibodies. When the immunoreactivity for the MKP-1 protein was compared with normal tissue as internal positive control, the intensity of staining identified three different groups of high-grade PIN lesions. The intensity was weak (+) in 56% (n = 28) of high-grade PIN lesions: in all these cases, a condensation of the staining beneath the apical portion of the cell membrane was observed (Fig. 2). Thirty percent (n = 15) of high-grade PIN cases showed intense (++) immunoreactivity for MKP-1 protein when compared to normal prostate (Fig. 3), whereas 14% (n = 7) of PIN cases were negative (–) (Fig. 4). In contrast, the cytoplasm of all the epithelial cells of high-grade PIN lesions consistently showed strong hybridisation signal (++) for MKP-1 mRNA (Figs. 3, and 4, Table 1).

The frequency of apoptotic cells (count per thousand cell or ‰) increased from PIN cases showing intense immune reactivity for MKP-1 protein (5.1 ± 3.4 SD ‰) to cases with weak negative for the protein (12.1 ± 5.0 SD ‰). The difference between high-grade PIN MKP-1 intensity (9.2 ± 3.5 SD ‰), to high-grade PIN with intense MKP-1 immunoreactivity and negative forms was found to be statistically significant (P < 0.008 by Bonferroni/Dunn's multiple comparison procedure; Table 2).

In prostatic adenocarcinoma surrounding PIN lesions JNK-1, ERK-1, and p38/RK protein expression was intense (++) in 80% of the adenocarcinomas and weak (+) in the remaining 20% (Table 1). The immunostaining for MKP-1 protein in prostate adenocarcinomas was intense (++) in 50%, weak (+) in 40% and negative (–) in 10% of the cases (Table 1). There was co-expression of MKP-1 protein and mRNA in any of tumours evaluated, as previously described [29] (Table 1).

Discussion

Prostatic carcinoma is the most common cancer in men in the United States, and its incidence is increasing [12, 39]. Although currently very little is known about the molecular mechanisms involved in the early phases of prostate cancer development, it is well accepted that

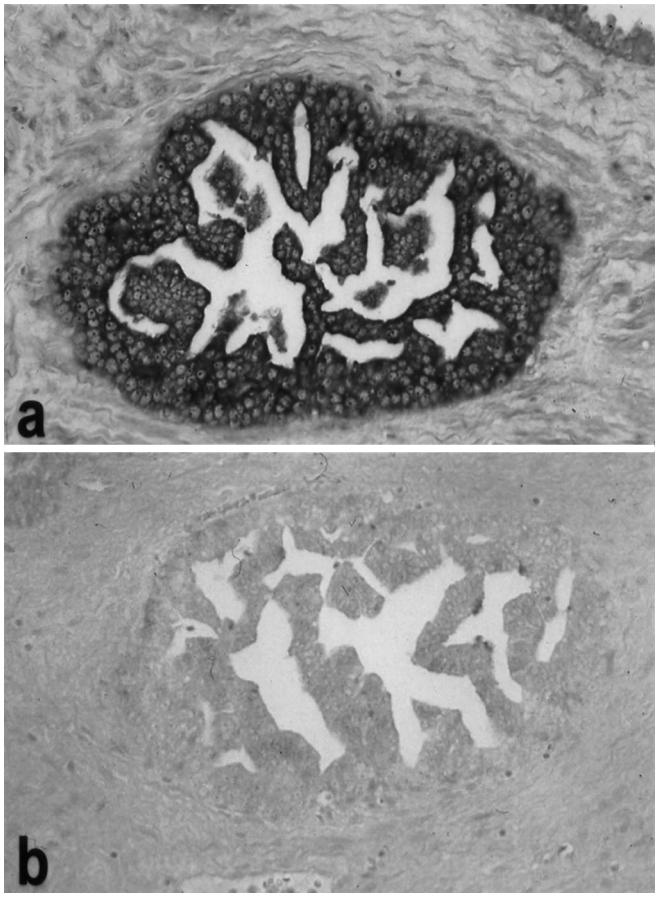

Fig. 4 **a** In situ expression of MKP-1 mRNA showing strong hybridisation signal in a high-grade PIN **b** displaying no immunoreactivity for MKP-1 protein. Original magnification × 200

high-grade prostatic intraepithelial neoplasia represents the direct precursor of prostate cancer [4, 10]. In contrast to hyperplastic epithelium, this premalignant lesion is genotypically and phenotypically similar to cancer [5, 10, 14, 36].

The growth regulation of stem cells and of their progeny and the homeostatic control between proliferative and nonproliferative behaviour are disrupted in PIN, leading to the expansion of the cell proliferating compartment and to a decrease in extent of the differentiated compartment. This was shown by Montironi et al. [33, 34] in studies in which the frequency and location of proliferation markers and of apoptotic bodies were analysed. In particular, the frequency of proliferating cells and of apoptotic bodies (ABs) was found to increase from normal prostate through PIN to adenocarcinoma. However, the AB-related values were approximately one-eighth to one-tenth of those obtained with proliferation markers.

The disrupted balance between cell proliferation and death observed in prostate oncogenesis probably derives from the accumulation of genetic changes modifying the expression or function of specific genes controlling cell proliferation, differentiation, and programmed cell death [5]. The MAP kinase pathways (extracellular signal-regulated protein kinases, c-jun N-terminal kinases/stress-activated protein kinases and re-activating kinases) represent a mechanism of signal transduction that, in part, affects the regulation of cell growth and cell death [11]. Which response prevails (to grow or to induce apoptosis) would depend, at least in part, on quantitative issues such as the nature of the stimulus, the density of receptors in the stimulatory and inhibitory pathways, and the intensity and duration of each kind of signal [13, 23, 37]. MAP kinases are activated by phosphorylation on tyrosine and threonine residues and inactivated by dual de-phosphorylation. In fact, MKP-1, a dual-specificity phosphatase, also inactivates ERKs [45], JNKs [16, 27] and p38/RK as well [2, 8, 16, 49]. Selective inhibition of the stress-activated cascade, however, is essential for mitogenic signals to induce a productive response.

Since all three parallel MAP kinase pathways are consistently activated in PIN, and MKP-1 expression is induced by the same signals that stimulate ERK and JNK activity [2, 3, 44], our results suggest that MKP-1 may differentially regulate the interaction or ′cross-talk′ between the MAP kinase pathways [16]. MKP-1 might be a key control point of the relative activities of these enzymes with similar biochemical functions, yet profoundly different physiological end-points. Previously we found that overexpression of both MKP-1 mRNA and protein occurs in breast, bladder, colon and prostate cancers, concomitantly with the expression of MAP kinases, such as ERK-1, ERK-2 and JNK-1. In addition, in prostate cancer, JNK-1, but not ERK-1, enzymatic activity seems to be inversely related to MKP-1 levels [28, 29]. These findings further support the hypothesis that MKP-1, even though it regulates ERKs via a short negative feedback loop [45], may preferentially dephosphorylate and thus inactivate JNKs in human tumours [1, 15, 16, 19, 27, 48]. In particular, because simultaneous activation of proliferative and apoptotic pathways results in conflicting signal to the cell, selective inhibition of apoptotic signals, possibly by MKP-1, appeared to be essential for mitogenic signals to induce a productive response.

The frequency of apoptotic cells was found to be increased from high-grade PIN cases showing intense immunoreactivity for MKP-1 protein to cases with weak MKP-1 intensity. In fact, the highest number of apoptotic cells was detected in high-grade PIN negative for the protein. The difference between cases with intense immunoreactivity for MKP-1 antibody and negative ones was found to be statistically significant. The inverse correlation between MKP-1 protein expression and the number of apoptotic cells supports the hypothesis of preferential inhibition by MKP-1 of the JNK pathway leading to apoptosis in PIN lesion, as previously suggested [19, 29].

The findings that ERK-1, JNK-1 kinases and MKP-1 mRNA are overexpressed in PIN compared with normal prostate are in agreement with the results of a previous study on epithelial carcinogenesis [28, 29]. It can be

speculated that MKP-1 may have a key role in the hormone- and/or growth factor-mediated proliferative response in PIN, and progression from preneoplastic to neoplastic lesion may be promoted by the continued enhancement of the growth factor/MAP kinase pathways.

Results supporting these findings were obtained in an experimental study conducted in Noble rats. Prostatic dysplasia, which is morphologically similar to human prostatic intraepithelial neoplasia, and carcinoma were induced with testosterone and oestradiol-17β treatment in the dorsolateral prostate [25]. As in human PIN, both ERK-1 and MKP-1 were strongly expressed only in dysplastic/PIN lesions. Those findings suggested that dual hormone treatment induces changes in the signal transduction pathways, which favour the protracted mitogenic action of MAP kinases. In particular, ERK-1 induction of cell proliferation and the concomitant suppression of apoptosis via inactivation of the JNK pathway by MKP-1 may function co-operatively in the early phases of experimental prostate carcinogenesis [16].

In conclusion, the data obtained in this study indicate that MAP kinase proteins are up-regulated in the early phases of prostatic carcinogenesis. Further, preferential inhibition of the "apoptotic kinases" JNK-1 and p38/RK by MKP-1 has been previously demonstrated [16, 19, 29, 49]. Here we showed an inverse correlation between MKP-1 expression and percent apoptosis in PIN. MKP-1 may thus shift the balance existing between cell proliferation and death by preferentially inhibiting intracellular regulators leading to programmed cell death.

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References

1. Alessi DR, Gomez N, Moorhead G, Lewis T, Keyse SM, Cohen P (1995) Inactivation of p42 MAP kinase by protein phasphatase 2A and a protein tyrosine phosphatase, but not CL100, in various cell lines. Curr Biol 5:283–295
2. Beltman J, McCormick F, Cook SJ (1996) The selective protein kinase C inhibitor, Ro-31–8220, inhibits mitogen-activated protein kinase phosphatase-1 (MKP-1) expression, induces c-jun expression, and activates jun N-terminal kinase. J Biol Chem 271:27018–27024
3. Bokemeyer D, Sorokin A, Yan M, Ahn NG, Templeton DJ, Dunn MJ (1996) Induction of mitogen-activated protein kinase phosphatase 1 by the stress-activated protein kinase signaling pathway but not by extracellular signal-regulated kinase in fibroblasts. J Biol Chem 271:639–642
4. Bostwick DG (1995) High grade prostatic intraepithelial neoplasia: the most likely precursor of prostatic cancer. Cancer 75:1823–1836
5. Bostwick DG, Pacelli A, Lopez-Beltran A (1996) Molecular biology of prostatic intraepithelial neoplasia. Prostate 29:117–134
6. Cano E, Hazzalin CA, Kardalinou E, Buckle RS, Mahadevan LC (1995) Neither ERK nor JNK/SAPK MAP kinase subtypes are essential for histone H3/HMG-14 phospholylation or c-fos and c-jun induction. J Cell Sci 108:3599–3609
7. Charles CH, Abler AS, Lau LF (1992) cDNA sequence of a growth factor-inducible early gene and characterization of the encoded protein. Oncogene 7:187–190
8. Chu Y, Solski PA, Khosravi-Far R, Der CJ, Kelly K (1996) The mitogen-activated protein kinase phosphatase PAC1, MKP-1, and MKP-2 have unique substrate specificities and reduced activity in vivo towards the ERK2 sevenmaker mutation. J Biol Chem 271:6497–6501
9. Cowley S, Paterson H, Kemp P, Marshall C (1994) Activation of MAP kinase kinase is necessary and sufficient for PC12 differentiation and for transformation of NIH3T3 cells. Cell 77:841–852
10. Davidson D, Bostwick DG, Qian J, Wallan PC, Oesterling JE, Rudders RA, Siroky M, Stilmant M (1995) Prostatic intraepithelial neoplasia is a risk factor for adenocarcinoma: predictive accuracy in needle biopsies. J Urol 154:1295–1299
11. Davis RJ (1995) Transcriptional regulation by MAP kinases. Mol Reprod Dev 42:459–467
12. Demers RY, Swanson GM, Weiss LK, Kau TY (1994) Increasing incidence of cancer of the prostate. The experience of black and white men in the Detroit metropolitan area. Arch Intern Med 154:1211–1216
13. Denhardt DT (1996) Signal-transducing protein phosphorylation cascades mediated by Ras/Rho proteins in the mammalian cell: the potential for multiplex signalling. Biochem J 318:729–747
14. Epstein JI (1995) Prostate biopsy interpretation. (Biopsy interpretation Series) Lippincott-Raven, Philadelphia
15. Franklin CC, Kraft AS (1995) Constitutively active MAP kinase kinase (MEK1) stimulates SAP kinase and c-jun transcriptional activity in U937 human leukemic cells. Oncogene 11:2365–2374
16. Franklin CC, Kraft AS (1997) Conditional expression of the Mitogen-activated Protein Kinase (MAPK) Phosphatase MKP-1 preferentially inhibits p38 MAPK and Stress-activated Protein Kinase in U937 cells. J Biol Chem 272: 16917–16923
17. Gould GW, Cuenda A, Thomson FJ, Cohen P (1995) The activation of distinct mitogen-activated protein kinase cascades is required for the stimulation of 2-deoxyglucose uptake by interleukin-1 and insulin-like growth factor-1 in KB cells. Biochem J 311:735–738
18. Han J, Lee J-D, Bibbs L, Ulevitch J (1994) A MAP Kinase targeted by endotoxin and hyperosmolarity in mammalian cells. Science 265:808–811
19. Hirsch DD, Stork PJ (1997) Mitogen-activated protein kinase phosphatases inactivate stress-activated protein kinase pathways in vivo. J Biol Chem 272:4568–4575
20. Ichijo H, Nishida E, Irie K, ten Dijke P, Saitoh M, Moriguchi T, Takagi M, Matsumoto K, Miyazono K, Gotoh Y (1997) Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathway. Science 275:90–94
21. Jiang Y, Chen C, Li Z, Guo W, Gegner JA, Lin S, Han J (1996) Characterization of the structure and function of a new mitogen-activated protein kinase (p38-beta). J Biol Chem 271: 17920–17926
22. Keyse SM, Emslie EA (1992) Oxidative stress and heat shock induce a human gene encoding a protein-tyrosine phosphatase. Nature 359:644–647
23. Krontiris TG (1995) Molecular Medicine: Oncogenes. N Engl J Med 333:303–306
24. Kyriakis JM, Banerjee P, Nikolakami E, Dai T, Rubie EA, Ahmad MF, Avruch J, Woodgett JR (1994) The stress-activated protein kinase subfamily of c-jun kinases. Nature 369:156–160
25. Leav I, Magi-Galluzzi C, Ziar J, Stork PJS, Ho S-M, Loda M (1996) Mitogen-activated protein kinase and mitogen-activated kinase phosphatase-1 expression in the Noble rat model of sex hormone-induced prostatic dysplasia and carcinoma. Lab Invest 75:361–370
26. Lee JC, Laydon JT, McDonnell PC (1994) A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. Nature 372:739–743
27. Liu Y, Gorospe M, Yang C, Holbrook NJ (1995) Role of mitogen-activated protein kinase phosphatase during the cellular response to genotoxic stress. J Biol Chem 270:8377–8380

28. Loda M, Capodieci P, Mishra R, Yao H, Corless C, Grigioni W, Wang Y, Magi-Galluzzi C, Stork PJS (1996) Expression of MAP kinase phosphatase-1 (MKP-1) in the early phases of human epithelial carcinogenesis. Am J Pathol 149:1553–1564
29. Magi-Galluzzi C, Mishra R, Fiorentino M, Montironi R, Yao H, Capodieci P, Wishnow K, Kaplan I, Stork PJS, Loda M (1997) MKP-1 is overexpressed in prostate cancers and is inversely related to apoptosis. Lab Invest 76:37–51
30. Marais R, Marshall CJ (1996) Cell signalling. (Cancer surveys, vol 27). Cold Spring Harbor Laboratory, Press, Cold Spring Harbor, pp 101–125
31. Marshall CJ (1995) Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. Cell 80:179–185
32. Misra-Press A, Rim CS, Yao H, Roberson MS, Stork PJS (1995) A novel mitogen-activated protein kinase phosphatase. J Biol Chem 24:14587–14596
33. Montironi R, Magi-Galluzzi C, Diamanti L, Giannulis I, Pisani E, Scarpelli M (1993) Prostatic intraepithelial neoplasia: expression and localization of proliferating cell nuclear antigen (PCNA) in epithelial, endothelial, and stromal nuclei. Virchows Arch [A] 422:185–192
34. Montironi R, Magi-Galluzzi C, Scarpelli M, Giannulis I, Diamanti L (1993) Occurrence of cell death (apoptosis) in prostatic intra-epithelial neoplasia. Virchows Arch [A] 423: 351–357
35. Montironi R, Magi-Galluzzi C, Muzzonigro G, Prete E, Polito M, Fabris G (1994) Effect of combination endocrine therapy (LHRH agonist and flutamide) in normal prostate, prostatic intraepithelial neoplasia and prostatic adenocarcinoma. J Clin Pathol 47:906
36. Nagle RB, Brawer MK, Kittelson J, Clark V (1991) Phenotypic relationship of prostatic intraepithelial neoplasia to invasive prostatic carcinoma. Am J Pathol 138:119–128
37. Nahas N, Molski TFP, Fernandez GA, Sha'afi RI (1996) Tyrosine phospholylation and activation of a new mitogen-activated protein (MAP)-kinase cascade in human neutrophils stimulated with various agonists. Biochem J 318:247–253
38. Noguchi T, Metz R, Chen L, Mattei MG, Carrasco D, Bravo R (1993) Structure, mapping and expression of erp, a growth factor-inducible gene encoding a nontransmembrane protein tyrosine phosphatase, and effect of erp on cell growth. Mol Cell Biol 13:5195–5205
39. Parker SL, Tong T, Bolden S, Wingo PA (1996) Cancer statistics, 1996. CA Cancer J Clin 65:5–27
40. Potter M (1972) Immunoglobulin-producing tumors and myeloma, proteins of mice. Physiol Rev 52:613–719
41. Raingeaud J, Gupta S, Rogers JS, Dickens M, Han J, Ulevitch RJ, Davis RJ (1995) Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated kinase activation by dual phosphorylation on tyrosine and threonine. J Biol Chem 270:7420–7426
42. Sells SF, Wood DPJr, Joshi-Barve SS, Muthukumar S, Jacob RJ, Crist SA, Humphreys S, Rangnekar VM (1994) Commonality of the gene programs induced by effectors of apoptosis in androgen-dependent and -indipendent prostate cells. Cell Growth Differ 5:457–466
43. Sluss HK, Barett T, Derijard B, Davis RJ (1994) Signal transduction by tumor necrosis factor mediated by JNK protein kinases. Mol Cell Biol 14:8376–8384
44. Sun H, Charles CH, Lau LF, Tonks NK (1993) MKP-1 (3CH134), an immediate early gene product, is a dual specificity phosphatase that dephosphorylate MAP kinase in vivo. Cell 75:487–493
45. Sun H, Tonks NK, Bar-Sagi D (1994) Inhibition of ras-induced DNA synthesis by expression of the phosphatase MKP-1. Science 266:285–288
46. Takano S, Fukuyama H, Fukumoto M, Hirashimizu K, Higuchi T, Takenawa J, Nakayama H, Kimura J, Fujita J (1995) Induction of CL100 protein tyrosine phosphatase following transient forebrain ischemia in the rat brain. J Cereb Blood Flow Metab 15:33–41
47. Waskiewicz AJ, Cooper JA (1995) Mitogen and stress response pathway: MAP kinase cascades and phosphatase regulation in mammals and yeast. Curr Opin Cell Biol 7:798–805
48. Wu J, Lau LF, Sturgill TW (1994) Rapid deactivation of MAP kinase in PC12 cells occurs independently of induction of phosphatase MKP-1. FEBS Lett 353:9–12
49. Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME (1995) Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 270:1326–1331