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Original contribution

Expression of store-operated channel components in prostate cancer: the prognostic paradox☆,☆☆

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Summary In vitro studies in prostate cancer (PCa) cell lines have suggested a key and complex role of the store-operated channels (SOCs) in major cancer hallmarks, including proliferation, apoptosis, and migration. In the present study, we investigated in vivo the expression of the SOC components transient receptor potential canonical (TRPC) 1, TRPC4, Orai1, and stromal interaction molecule 1 (STIM1), during all stages of PCa progression, and evaluated their prognostic impact in clinically localized cancer (CLC). The expressions of TRPC1, TRPC4, Orai1, STIM1, and the androgen receptor and the proliferation marker Ki-67 were evaluated by immunohistochemistry on tissue microarrays containing samples of normal prostate tissues (n = 91), prostatic intraepithelial neoplasia (n = 61), CLC surgically treated (n = 238), and castration-resistant prostate cancer (CRPC; n = 45). All markers significantly increased in CLC compared with normal tissues and (for Orai1 and STIM1) in advanced pT3 tumors compared with pT2. In contrast, their expression decreased in CRPC, particularly for Orai1. In CLC, staining for TRPC1, Orai1 and STIM1 correlated with androgen receptor expression, and TRPC1 status was associated with lower proliferation and longer recurrence-free survival, after adjusting for classical prognostic markers. Although increased SOC expression during PCa progression supports a role in cancer cell migration, the inverse association between TRPC1 and biochemical relapse suggests a protective effect in CLC. Moreover, the dramatic down-regulation of Orai1 in CRPC supports its role in apoptosis at this stage of the disease. These results call for caution when considering SOCs as potential therapeutic targets for PCa.

1. Introduction

Changes in cytosolic Ca^{2+} trigger events are critical for cancer development and progression, such as proliferation, apoptosis, and migration. Intracellular Ca^{2+} increase is due to either influx from the extracellular medium or release of intracellular stores, mainly from the endoplasmic reticulum (ER). Store-operated calcium entry (SOCE) is mediated by

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store-operated channels (SOCs) that are localized at the plasma membrane and activated by ER Ca^{2+} store depletion [1,2]. The molecular components of SOCs identified to date are Orai channels, some transient receptor potential canonical (TRPC) channels such as TRPC1 and TRPC4, and the ER partner stromal interaction molecule 1 (STIM1). SOCs that require STIM1 and Orai1 for operation are the major Ca^{2+} entry pathway in nonexcitable cells [1,2]. STIM1 is a single-transmembrane domain protein that is mostly localized to ER membrane. After a decrease in the luminal Ca^{2+} concentration, STIM1 translocates into punctuae close to the plasma membrane. Herein, STIM1 has been shown to gate and activate 2 Ca^{2+}-permeable channels, namely, TRPC1 and Orai1 [3]. Moreover, TRPC1 has also been suggested to interact with Orai1. The regulation of TRPC1 by STIM1 was extended to other TRPC channels, mainly TRPC4 [4]. It is therefore likely that STIM1/Orai1/TRPCs may function in vivo as complexes that mediate SOC activity.

Prostate cancer (PCa) is the most frequent noncutaneous malignancy in men older than 50 years. Pathways that trigger PCa progression are related to cell migration, proliferation, and resistance to apoptosis. In PCa cells, TRPC1 and TRPC4 have been shown to be major contributors of SOC activation by the P2Y agonist ATP, leading to decreased proliferation, which is in contrast increased by TRPC6 [5]. Moreover, it has been suggested that in the PCa cell line LNCaP, the functional coupling of STIM1 to Orai1 is required for proapoptotic effects [6]. In contrast, the couple Orai1/STIM1 is involved in both the migration and metastatic potential of some cancer cell lines [7], although this effect has not yet been demonstrated in PCa cells. Thus, 2 main cancer hallmarks, migration and resistance to apoptosis, seem to require the opposite expression of STIM1/Orai1-based SOCE.

Despite these results obtained in vitro, the protein expression of the ion channels TRPC1/C4, Orai1, and STIM1 has never been evaluated in vivo in human PCa.

In the present study, we aimed to analyze the expression of SOC components during all the stages of PCa natural history, in association with markers of aggressiveness and recurrence after treatment.

2. Materials and methods

2.1. Patients and samples

Some tissue samples analyzed in this study have already been used to assess ERG expression, in order to detect *TMPRSS2*/*ERG* fusion in PCa [8].

Normal-appearing prostate tissues (n = 91), high-grade prostatic intraepithelial neoplasia (PIN; n = 61), and clinically localized cancer (CLC) samples (n = 238) were obtained from white patients treated by radical prostatectomy for localized PCa at Tours University Hospital and Institut Mutualiste Montsouris. Normal tissue was taken in the peripheral zone, in areas distant from cancer, and high-grade PIN in areas adjacent to cancer.

CLC cases were composed of 119 tumors with negative surgical margins and biochemical relapse (defined as 2 consecutive increases in serum prostate-specific antigen [PSA] ≥0.2 ng/mL), matched with 119 tumors without recurrence after at least 4 years of follow-up. Each patient with biochemical relapse was matched with 1 patient with identical age group, preoperative PSA, Gleason score, and pathological stage, but who was free of recurrence after at least the same follow-up. This matching allows to have already taken into account the traditional predictive markers, in order to analyze the prognostic value of the candidate genes. The median time to recurrence was 20 months (range, 3-90 months), and the median follow-up in the groups of patients without recurrence was 86 months (range, 48-128 months). In case of multifocal cancer, the dominant nodule (ie, the one with the highest Gleason score) was sampled. None of the patients received androgen ablation therapy before surgery.

Forty-five cases of castration-resistant prostate cancers (CRPCs) were selected from patients treated with exclusive androgen deprivation therapy (ADT). Patients were selected when they initially responded to exclusive ADT and had post–hormonal relapse tissue sample suitable for analysis. Hormonal relapse was defined as 2 consecutive rises in PSA, with serum testosterone lower than a castration level of 50 ng/dL. Tissues were collected by transurethral resection, performed because of lower urinary tract symptoms associated with local tumor progression.

The characteristics of patients and tissues are summarized in Table 1. Written informed consents were obtained from patients in accordance with the requirements of the medical ethic committee of our institutes.

Table 1 Patients and tissues characteristics

Groups	NL (n = 91)	PIN (n = 61)	CLC (n = 238)	CRPC (n = 45)
Age (y), median (range)	62 (46-71)	62 (46-71)	63 (46-75)	72 (56-86)
PSA (ng/mL)	–	–	9.1 (1.5-23)	12.5 (0.2-285)
pTNM				
pT2	NA	NA	152	NA
pT3			86	
Gleason score				
6	NA	NA	58	NA
7 (3 + 4)			66	
7 (4 + 3)			93	
≥8			21	

Abbreviations: NL, normal prostate tissue; PIN, high-grade prostatic intraepithelial neoplasia; CLC, clinically localized cancer; CRPC, castration-resistant prostate cancer; PSA, prostate-specific antigen; NA, not applicable.