Specific Properties of a C-terminal Truncated Androgen Receptor Detected in Hormone Refractory Prostate Cancer

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Summary Mutations in the human androgen receptor (AR) gene that lead to C-terminus truncated AR variants are frequently detected in prostate cancer (PC). These AR variants lack both the ligand-binding domain (LBD) and the AF-2 region. The aim of this study was to delineate the alternative mechanisms that lead to the activation of such AR variants as they are unresponsive to hormone stimulation, and to outline consequences of the loss of the LBD/AF-2 region on their functional properties. By using an MMTV-luciferase reporter construct and LY294002, U0126, or ZD1839, inhibitor of PI3K, MEK1/2, and EGFR signaling pathway respectively, we demonstrated that phosphorylation was required for full transcriptional activities of one these AR variants, the Q640X mutant AR. Western-blot analyses confirmed that these inhibitors affect the phosphorylation status of this AR variant. Furthermore, studies of the intranuclear colocalization of the Q640X AR with cofactors, such as CBP, GRIP-1, and c-Jun, reveal that the transcriptional complex that forms around the mutant AR is different to that formed around the wild type AR. We demonstrated that CBP and c-Jun are highly recruited by the mutant AR, and this leads to an unexpected activation of AP-1, NFAT, and NFκB transcriptional activities. Similar enhanced activities of these transcription factors were not observed with the wild type AR. The importance of the LBD/AF-2 for the regulation of AR transcriptional activities, the impact of the presence of such AR variants on PC cells proliferation and survival, and on progression to androgen independence are discussed.

Introduction

Androgen receptor (AR) mutations are recurrent events during the progression of prostate cancer (PC) to a hormone-refractory status. Selected AR mutations confer new functional properties to the AR, and favor PC cell growth and survival in an androgen-depleted environment. Mutations in the ligand-binding domain (LBD) and the activation function-2 (AF-2) of the AR have been shown to affect receptor activation. Indeed, the C-terminal end (CTE), including the LBD and AF-2 regions, is essential for AR regulation notably being the target of direct or indirect phosphorylation.
after ligand-binding or by interaction with other signal transduction pathways (1, 2). The control of the AR transcriptional activities is also upon the control of numerous cofactors. The AR requires interaction with cofactors to activate (coactivators) or to inhibit (corepressor) target genes (3). The cAMP response element binding protein (CREB)-binding protein (CBP)/p300, is one of these coactivators that interplay with the AR at the target gene promoter to facilitate DNA occupancy, chromatin remodeling, or the recruitment of general transcription factors associated with the RNA polymerase II (3). Also, CBP being a partner for other transcription factors allows crosstalk of the AR with other signaling pathways. Also, the glucocorticoid receptor interacting protein-1 (GRIP-1), coactivator of the AR, induces the appropriate folding of the AR or facilitates AR N/C-terminal interactions to direct target gene expression. Similarly, the proto-oncoprotein c-Jun acts as an AR coactivator by enhancing AR N/C-terminal interactions (3, 4). In summary, the CTE is the target of many regulation pathways and the loss of these parts of AR should be significant.

In clinical studies, we detected several nonsense mutations in AR gene of localized and metastatic PC patients that lead to C-terminal truncated AR proteins. One of these mutations, the Q640X AR variant, has been previously described as a ligand-independent and constitutive transcriptional factor (Fig. 1) (5). In this study, we demonstrate that the Q640X AR requires interactions with other signal transduction pathways to be fully active. Moreover, we show that the lack of CTE leads to a differential recruitment of cofactors and then could affect remarkably target gene involved in cell proliferation, survival, and differentiation.

Results

Activation of the Q640X AR Variant Needs Phosphorylation. The AR is a phosphoprotein in which the serine 16, 81, 94, 256, 308, 424, 650, and 791 are phosphorylated (6). The phosphorylation of the serine 650 is activated by the epidermal growth factor receptor (EGFR) pathway (1), although serine 213 and 791 are regulated in vitro by PI3K pathway (7, 8). Moreover, the phosphorylation of serine 514 requires the MEK1/2 pathway and regulates the AR-induced cytotoxicity.