RHO GTPASES AND PROSTATE CANCER PROGRESSION
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INTRODUCTION. Most prostate cancer initially displays androgen dependency for growth and survival such that tumor growth is inhibited by androgen deprivation. However, prostate cancer invariably progresses to an androgen-independent state marked by growth that is unaffected by hormonal manipulation. Abnormally suppressed levels of cyclin-dependent kinase inhibitors (CKIs) may contribute to deregulated cell proliferation in androgen-independent cancer. The androgen-independent human prostate cancer cell lines, LNCaP-R1, ALVA31, and PC-3, express low levels of the CKI, p21<sub>CIP1</sub>, compared to the less malignant, androgen-dependent LNCaP cells (1). Rho GTPases, including RhoA, Rac1 and Cdc42, are signaling proteins which are involved in a number of cellular processes including cell cycle progression, at least in part through regulation of CKIs (reviewed in 2). We examined a possible role for Rho family signaling in regulating cell cycle progression and tumorigenesis in prostate cancer cells.

METHODS. This investigation utilized several prostate cancer cell lines, including the androgen-dependent LNCaP cell line as well as three androgen-independent cell lines, LNCaP-R1 (derived following androgen deprivation of LNCaP), ALVA31 and PC3. Experiments conducted include Rho GTPase “pull-down” activity assays, Western and Northern blot analyses, in vitro kinase assays, <sup>3</sup>H-thymidine uptake assays and xenograft studies in nude mice.

RESULTS. To determine the relationship between Rho family signaling and p21 regulation, we inhibited Rac1, Cdc42 and RhoA using toxin inhibitors, overexpression of Rho effector binding domains and dominant negative Rho mutants. Inhibition of RhoA or Rac1 resulted in upregulation of p21 mRNA and protein in all of the androgen-independent cell lines whereas p21 levels were unchanged in the androgen-dependent LNCaP cells (3). Levels of cyclin D were not affected by inhibition of Rho proteins. Inhibition of Cdc42 had no effect on
p21 in any of the cell lines. In complementary studies, introduction of constitutively active Rac1 resulted in down regulation of p21 in LNCaP cells. Pull-down assays using Rho effector binding domains were conducted to determine activity levels of the three well-characterized Rho family members in androgen-dependent and –independent cell lines. Rac1 activity but not Rac1 protein levels were elevated in the androgen-independent cells compared to LNCaP cells. RhoA activity was comparable but relatively low in the four cell lines while Cdc42 activity was negligible. Inhibition of Rac1 or RhoA not only led to elevated p21 in androgen-independent cell lines but also inhibited cyclin-dependent kinase 2 activity and decreased cell proliferation (3). Initial studies using dominant negative mutants to define the downstream effectors of this response revealed that Rho-kinase and LIM kinase participate in RhoA suppression of p21 levels in androgen-independent prostate cancer cell lines. To assess the role of Rac1 in tumorigenesis, we stably expressed constitutively active Rac1 in LNCaP cells (LNCaP/CA-Rac1) and assessed tumor formation in nude mice. LNCaP/CA-Rac1 xenografts exhibited a higher tumor take rate and grew more rapidly than LNCaP controls expressing the vector alone (LNCaP/neo).

DISCUSSION. We defined roles for RhoA and Rac1 GTPases in the suppression of p21 leading to uncontrolled cell growth in the context of prostate cancer progression. The RhoA effects were mediated by Rho kinase and LIM kinase but Rac1 downstream effector(s) have not yet been determined. Rac1 hyperactivity in androgen-independent cells may contribute to the aggressive phenotype of these cells, as its introduction was sufficient for malignant conversion of otherwise indolent LNCaP cells. These results reveal Rho GTPases and associated effector molecules as potential targets against androgen independent prostate cancer growth and progression.

ACKNOWLEDGEMENT. This work is supported by the U.S. Department of Defense DAMD 17-02-1-0094.

REFERENCES.