# Marine compound rhizochalinin shows high *in vitro* and *in vivo* efficacy in castration resistant prostate cancer

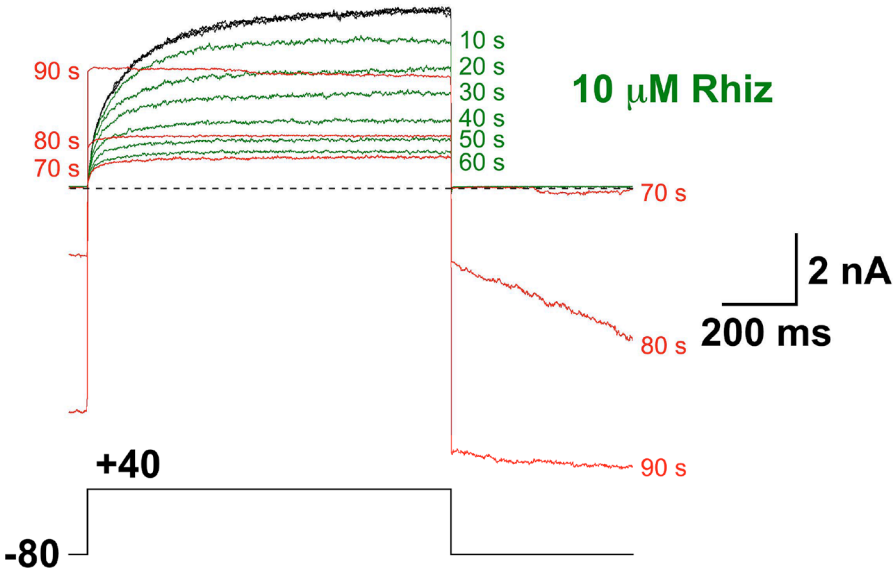
## Supplementary Materials

### Supplementary Table S1: List of antibodies used

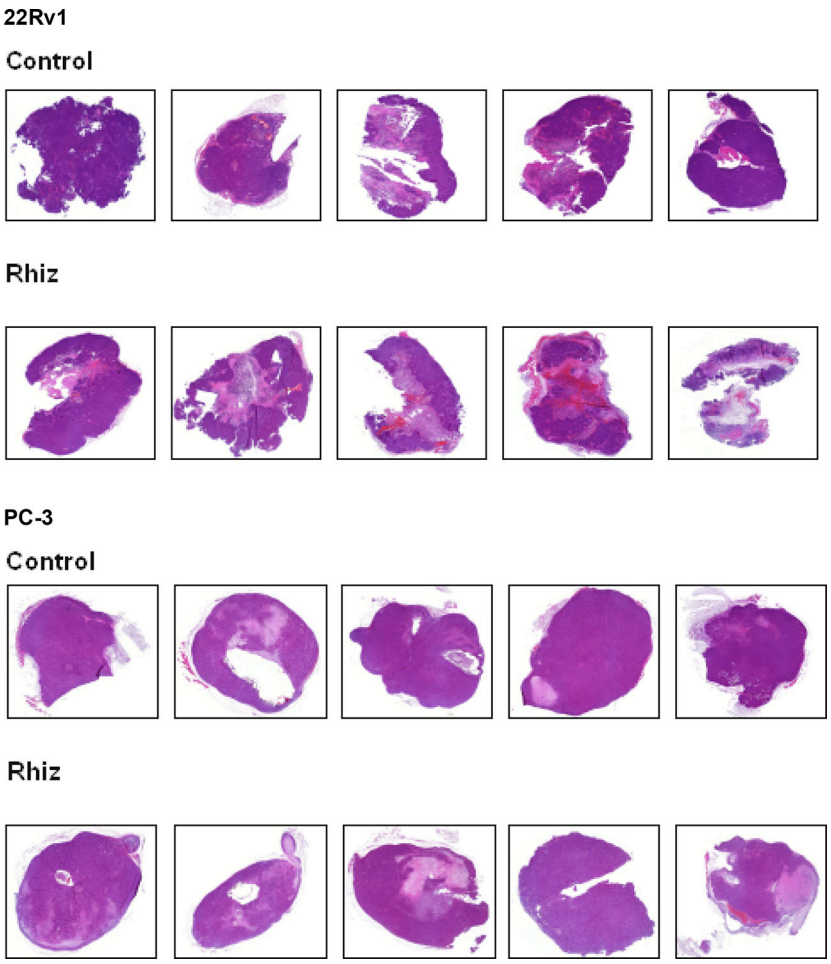
| Antibodies | Clonality | Source | Cat.-No. | Used concentration | Manufacturer |
|---|---|---|---|---|---|
| anti-α-Tubulin | mAb | mouse | T5168 | 1:5000 | Sigma-Aldrich |
| anti-β-Actin-HRP | pAb | goat | sc-1616 | 1:10000 | Santa Cruz |
| anti-AR | pAb | rabbit | sc-816 | 1:200 | Santa Cruz |
| anti-AR-V7 | mAb | rabbit | 198394 | 1:1000 | abcam |
| anti-Bad | mAb | rabbit | #9239 | 1:1000 | Cell Signaling |
| anti-Bax | mAb | rabbit | #5023 | 1:1000 | Cell Signaling |
| anti-Bcl-2 | pAb | rabbit | #2876 | 1:1000 | Cell Signaling |
| anti-GAPDH | mAb | mouse | G041 | 1:10000 | Abm |
| anti-Caspase-8 | mAb | mouse | #9746 | 1:1000 | Cell Signaling |
| anti-Caspase-9 | pAb | rabbit | sc-8355 | 1:200 | Santa Cruz |
| anti-cleaved Caspase-3 | mAb | rabbit | #9664 | 1:1000 | Cell Signaling |
| anti-IGF-1 | mAb | mouse | sc-74116 | 1:500 | Santa Cruz |
| anti-LC3B-I/II | pAb | rabbit | #2775 | 1:1000 | Cell Signaling |
| anti-mTOR | mAb | rabbit | #2983 | 1:1000 | Cell Signaling |
| anti-p21$^{Waf1/Cip1}$ | mAb | rabbit | #2947 | 1:1000 | Cell Signaling |
| anti-p53 | mAb | mouse | #2524 | 1:1000 | Cell Signaling |
| anti-PAK1 | pAb | rabbit | #2602 | 1:1000 | Cell Signaling |
| anti-PARP | pAb | rabbit | #9542 | 1:1000 | Cell Signaling |
| anti-phospho-mTOR | mAb | rabbit | #5536 | 1:1000 | Cell Signaling |
| anti-PTEN | mAb | mouse | 04-035 | 1:1000 | Millipore |
| anti-Survivin | pAb | rabbit | NB500-201 | 1:1000 | Novus |
| anti-rabbit IgG-Alexa Fluor® 488 | | goat | #4412 | 1:1000 | Cell Signaling |
| anti-goat IgG-HRP | | rabbit | #31433 | 1:10000 | Thermo Scientific |
| anti-mouse IgG-HRP | | sheep | NXA931 | 1:10000 | GE Healthcare |
| anti-rabbit IgG-HRP | | goat | #7074 | 1:5000 | Cell Signaling |
| anti-rat IgG-HRP | | rabbit | ab6734-1 | 1:5000 | abcam |

## Supplementary Table S2: List of primers used

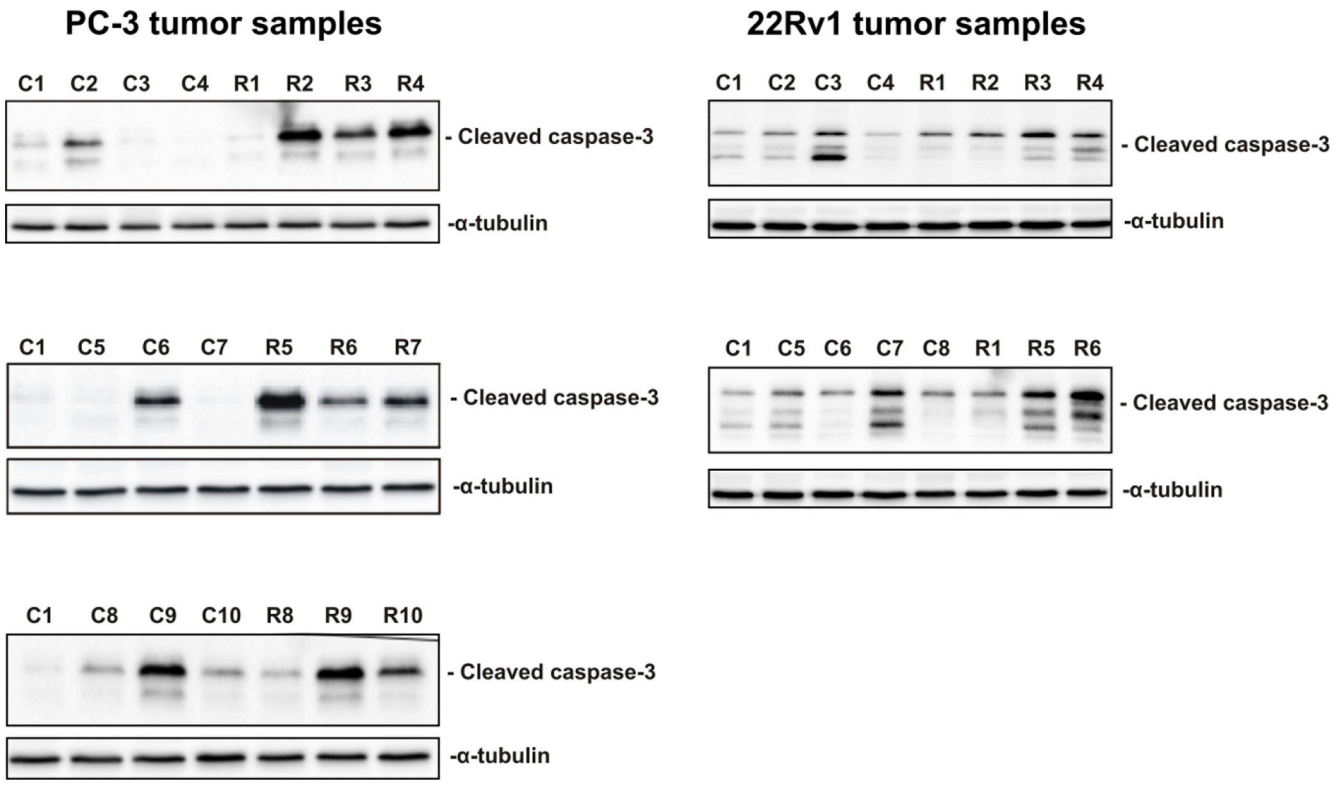
| Primer name | Primer sequence |
|---|---|
| AR-V7 forward | 5′-CCA TCT TGT CGT CTT CGG AAA TGT TA-3′ |
| AR-V7 reverse | 5′-TTT GAA TGA GGC AAG TCA GCC TTT CT-3′ |
| PSA forward | 5′-CAT CAG GAA CAA AAG CGT GA-3′ |
| PSA reverse | 5′-ATA TCG TAG AGC GGG TGT GG-3′ |
| IGF-1 forward | 5′-AGG AAG TAC ATT TGA AGA ACG CAA GT-3′ |
| IGF-1 reverse | 5′-CCT GCG GTG GCA TGT CA-3′ |
| GAPDH forward | 5′-TGC ACC ACC AAC TGC TTA-3′ |
| GAPDH reverse | 5′-GAG GCA GGG ATG ATG TTC-3′ |




**Supplementary Figure S1: Effect of Rhiz on heag1-mediated currents in a CHO cell.** Currents in response to repeated depolarizing voltage jumps (pulse protocol below traces) were recorded with the whole-cell patch-clamp technique from a transiently transfected CHO cell expressing heag1 channels (3 superimposed black traces: control bath solution, colored traces: bath solution containing 10 μM Rhiz). Time after the start of Rhiz application is indicated. During the first 60 s onset of current inhibition is observed (green traces), which is then accompanied by considerable leak current development (zero current indicated by dotted line) and eventually loss of membrane integrity (red traces).




**Supplementary Figure S2: H&E staining of the tumor sections.** Analysis of H&E stained tumor sections was performed by two independent blinded investigators for 5 tumors from each group (two biggest tumors + two smallest tumors + one middle-sized tumor from each group in both experiments). The quantification of necrotic-apoptotic area was performed using the Image J Software (NIH, Bethesda, USA).




**Supplementary Figure S3: Western blotting analysis of cleaved caspase-3 in the tumors.** The tumor samples were homogenized and analyzed as described in the Materials and methods. The quantification of the signal was performed using the Image J Software (NIH, Bethesda, USA). The level of cleaved caspase-3 was normalized against loading control. The signals within each membrane were normalized against the same control sample (which was loaded on each gel) and therefore were compared within the different membranes and then compared. "C" – the sample from the control group, "R" – the sample from the Rhiz-treated group.