Supplementary Materials

ERG IHC Methods

We used a BioGenex i6000 automated staining platform (BioGenex Laboratories Inc., Fremont CA). Five-μm sections of each tissue microarray were deparaffinized in xylene, followed by a graded alcohol rehydration. Antigen retrieval was performed by microwaving the tissue in citrate buffer for five minutes. ERG antisera (Clone ID: EPR3864, Epitomics, Inc., Burlingame, CA) was applied at 1:100 for one hour. Detection of the primary ERG antibody was carried out using the BioGenex SS Multilink secondary antibody, followed by horseradish peroxidase (HRP) conjugation to the secondary antibody using the Biogenex SS HRP Labeling kit. Visualization of ERG was accomplished using the DAB substrate kit (Vector Laboratories Inc., Burlingame, CA). Sections were subsequently counterstained with hematoxylin, and the sections were dehydrated in a graded series of alcohol prior to coverslip application. Tumor specimens were analyzed for ERG expression by a study pathologist. For all cases, the presence of ERG staining in the vasculature endothelium served as a positive internal control, and subsequent assessment of ERG was restricted to cores in which the positive internal control was observed. A case was called positive for ERG expression (i.e. ERG overexpression) if at least one core from an individual case had positive ERG staining observed within prostate cancer epithelial cells. Of cases positive for ERG on at least one core, 85% stained positive for ERG in all cores evaluated. When ERG status could not be assessed due to lack of remaining tumor tissue or negative internal endothelial control, sections of the original tumor blocks were re-stained for ERG.

PTEN IHC Methods

PTEN immunohistochemistry was performed manually on 11 out of 13 TMAs as previously described [6]. In brief, 4 μm biopsy sections were deparaffinized and re-hydrated under standard conditions. Antigen unmasking was performed by steaming in EDTA buffer (pH 8.0) for 45 minutes. Endogenous peroxidase activity was quenched by incubation with peroxidase block for 5 minutes at

room temperature. Slides were incubated for 45 minutes at room temperature with a rabbit anti-human PTEN antibody (Clone D4.3 XP; Cell Signaling, Danvers, MA; 1:50 dilution). A horseradish peroxidase-labeled polymer (PowerVision, Leica Microsystems, Bannockburn IL) was applied for 30 minutes at RT and signal detection was performed using 3,3'-diaminobenzidine tetrahydrochloride (DAB) as the chromagen. Slides were counterstained with hematoxylin, dehydrated, and mounted.

On a subset of two TMAs from the HPFS cohort, PTEN IHC was performed on the Ventana automated staining platform. To validate the protocol on this platform, we examined an additional TMA containing 50 cases with known PTEN protein status by manual staining and found 100% concordance between the PTEN protein status on the manual and automated platforms. The automated platform utilized CC1 antigen retrieval buffer (Ventana) for 32 minutes at 100°C, followed by incubation with the same antibody as the manual platform (above) at a 1:50 dilution at 36°C for 32 minutes, followed by the Optiview HRP multimer secondary detection system.

After staining, all TMAs were scanned at 20x magnification (Aperio) and segmented into TMAJ for scoring (http://tmaj.pathology.jhmi.edu/). PTEN immunohistochemistry was blindly scored by a trained uropathologist (TLL) using a previously validated dichotomous scoring system [6]. A tissue core was considered to have PTEN protein loss if the intensity of cytoplasmic and nuclear staining was markedly decreased or entirely negative across >10% of tumor cells compared to surrounding benign glands and/or stroma, which provide internal positive controls for PTEN protein expression. Markedly decreased staining for PTEN in tumor epithelium is defined in comparison to benign glands and stroma which provide internal positive controls for intact PTEN immunostaining in each tissue sample. In the vast majority of cases, this corresponds to 0+ intensity staining (Figure 1), or in a minority, very focal 1+ staining in tumor epithelium in a minority of cases if this staining is convincingly markedly different from internal benign glands. This simple dichotomous scoring system has been shown to be highly correlated with underlying homozygous genetic deletion of *PTEN* (Reference 6) in prior studies by SNP array. More recently, we have shown that intact PTEN protein by this assay is 91% specific for intact *PTEN* gene alleles and 98% sensitive for homozygous *PTEN* deletion in

comparison to a four-color DNA fluorescence *in situ* hybridization (FISH) assay. Given the importance of comparison to internal benign glands and/or stromal tissue staining, if there is inadequate internal positive control staining in a given tumor spot, the core is not scored. In addition, we have tested the effect of pre-analytic variables (tissue fixation method and duration, tissue processing technique, slide and block storage method and age) on PTEN immunohistochemistry and found these have minimal effects on staining characteristics or PTEN status calls in general.

On a subset of 6 of the 13 TMAs (including 2 with automated and 4 with manual staining) all cores were blindly and independently scored by a second pathologist (CLM) to check for inter-observer variability. On these 6 TMAs, PTEN scores were concordant in 98.4% of cores evaluated by both pathologists (925/940; including a total of 240 with PTEN loss identified by both reviewers), corresponding to a kappa statistic of 0.984. There was no significant difference between inter-observer variability on the TMAs stained on the manual or automated staining platforms.