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OMIP-040: Optimized Gating of Human Prostate Cellular Subpopulations

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Purpose and Appropriate Sample Types

This panel was optimized to quantify the relative frequency of the major cell types present in the human prostate in addition to their sorting for downstream applications. Tissue resident white blood cells (referred to here as leukocytes) are identified by CD45, epithelia are identified by CD326, and stroma are double negative with those markers. Epithelia can be further segregated into basal, luminal, and "other" populations using CD26 and CD271. Fibromuscular stroma can be identified by removing CD31-positive endothelia from the stroma. This panel can also serve as a backbone for the addition of markers to interrogate subpopulations within these major cell types. The panel has been validated on freshly digested and cryopreserved human prostate cells collected from young organ donors, BPH patients, and prostate cancer patients. Other tissue types have not been tested. Other basal cell markers including CD49f, podoplanin, and CD104 are tested and compared with CD271.

Background

The human prostate comprised pseudostratified epithelial glands, which are primarily made up of secretory tall columnar luminal epithelial cells and cuboidal basal epithelial cells. There are other epithelial cell populations, such as low frequency neuroendocrine cells, epithelial progenitors, and "other" epithelial cells that do not fall within the traditional basal and luminal cell gating schema. Glands are surrounded by a fibromuscular stroma, containing a heterogeneous mix of smooth muscle, fibroblasts, and nerves. Prostate tissue is also highly vascular and contains a high amount of infiltrating leukocytes in the diseased state (1). The cells of origin in prostate diseases are the subject of intense debate, but the antibodies used to identify various epithelial subpopulations are still unsettled (2–10). We therefore set out to optimize the gating strategy for human prostate cellular subpopulations (Table 1).

The pan-epithelial marker CD326 (EpCAM) along with the pan-leukocyte maker, CD45 is utilized to separate the three major cellular populations in the human prostate: leukocytes, epithelia, and stroma (Fig. 1F). Endothelial cells are removed from the stromal gate with CD31, leaving the heterogeneous fibromuscular population as a negative gate (Fig. 1H). Epithelial populations are divided using the basal marker CD271 and the luminal marker CD26 (Fig. 1G). These markers split epithelia into basal, luminal, and double negative "other" populations. CD49f is the most commonly used basal marker, but it has several limitations, which CD271 resolves. CD49f is a trimodal spectrum marker, basal epithelia are $CD49f^{Hi}$, luminal epithelia are $CD49f^{Lo}$, and a subpopulation of stroma are $CD49f^{Neg}$. This means that fluorescence-minus-one (FMO) experiments are not useful for objectively separating basal and luminal epithelial populations. This is why many investigators add CD26 to their panels (11–14) as a marker of luminal epithelia. This greatly improves the sorting, except that there is still an arbitrary separation between $CD26^{Neg}/CD49f^{Hi}$ basal and $CD26^{Neg}/CD49f^{Lo}$ "other" epithelia. The "other" epithelial population is not well defined at the molecular or cellular

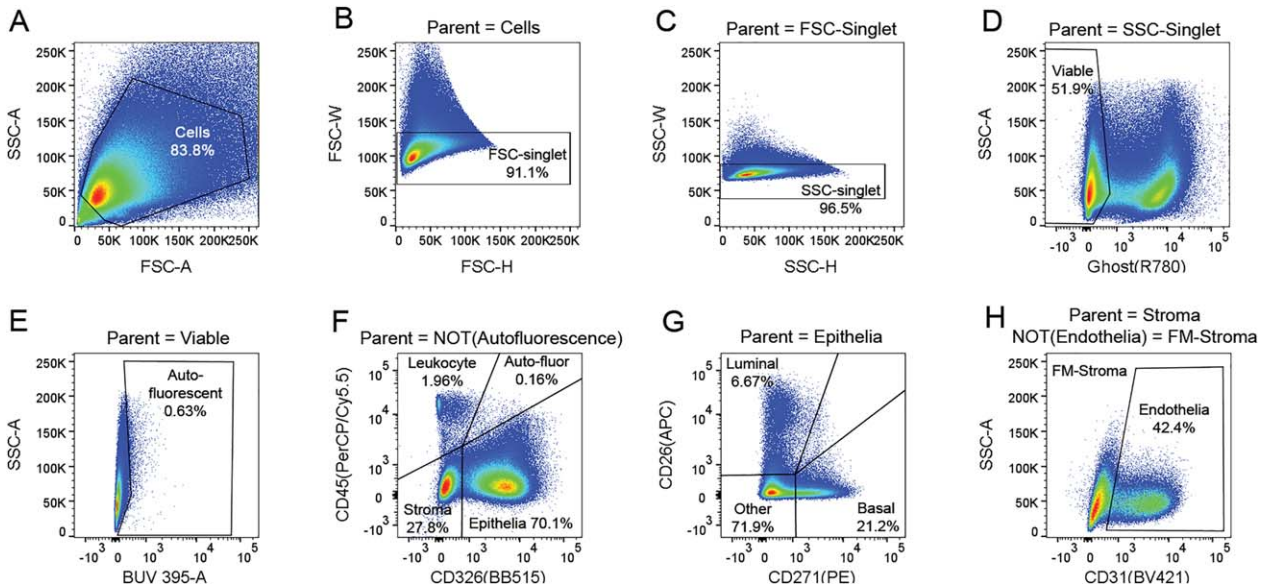

Figure 1. Gating strategy. Primary cells from a human prostate obtained from a 24-year-old male organ donor were stained with the antibodies listed in Table 2. This strategy removes doublets, low viability cells, and autofluorescent events (**A**–**E**). The panel identifies leukocytes, epithelia, and stroma (**F**), separates epithelia into basal, luminal, and "other" populations (**G**), and removes endothelial from the fibromuscular stroma gate (**H**).

level. It is currently unclear if these cells are intermediate between basal and luminal epithelia, a unique cell type, or a heterogeneous population of epithelial cell types. We performed a screen of cell-surface markers in a pool of cells labeled with CD26 and CD49f, and several markers were determined to be basal-specific. ITGB4 (CD104), NGFR (CD271), and podoplanin (PDPN) were highly enriched in basal epithelia, which were defined as $CD326^{Pos}/CD45^{Neg}/CD49f^{Hi}/CD26^{Neg}$ cells (data not shown). CD271, CD104, and PDPN appear to be bimodal, with luminal and "other" epithelia staining negative. They are spectrum markers between basal and "other" epithelia. The bimodality of these markers means that FMO experiments can now objectively be used to define positive (basal) and negative (other) cells. CD271 showed a slightly better separation between positive and negative cells than CD104 and podoplanin, which resulted in its choice for this panel. All of these markers have been shown to be basal epithelial markers to some extent previously (15–18). Specificity of CD271 and the other basal markers compared to CD49f are detailed in Figure S4.

Digested primary prostate cells are highly complex and exhibit a surprising amount of autofluorescence. This is especially evident when a surgical technique such as transurethral resection of the prostate (TURP) is used, which cauterizes the tissue and results in lower viability than other surgical techniques. These low viability cells exhibit a high amount of autofluorescence. This requires, at minimum, the use of an amine-reactive viability dye to exclude dead and dying cells (Fig. 1D), which accounts for some of the autofluorescent events. Autofluorescence is further complicated by the fact that secretory luminal epithelia can bind antibodies nonspecifically. Autofluorescent cells emit brightest when excited by high-energy lasers. In this panel, BUV395 was not utilized and, as a result, any events that stain brightly in that channel are considered autofluorescent (Fig. 1E). If a UV laser is not available, any empty channel can be used. Failing that, $BV421^{Pos}$ (CD31 in this panel) should contain the majority of autofluorescent events. Biologically, CD31 has not been found to be expressed in epithelia and its expression by carcinomas is very rare (19). This means that any events that are $CD31^{Pos}/CD326^{Pos}$ are most likely to be autofluorescent, and can be gated out.

All antibodies have been titrated to optimize the resolution of populations while maximizing antibody dilution and gates have been confirmed with FMO experiments as detailed in Figure S2.

Table 1. Summary table for OMIP-040

Purpose	Quantification and characterization of the major cell populations in primary human prostate tissues via cell surface markers
Species	Human
Cell types	Freshly digested and cryopreserved primary prostate cells from adult men
Cross-references	na

Table 2. Reagents used for OMIP-040

SPECIFICITY	CLONE	FLUOROCHROME	PURPOSE
		Ghost R780	Viability
CD45	H130	PerCP/Cy5.5	Leukocyte
CD326	EBA-1	BB515	Epithelia
CD271	ME20.4	PE	Basal epithelia
CD26	BA5b	APC	Luminal epithelia
CD31	WM59	BV421	Endothelia

Human Subjects

Healthy prostates were collected from young male organ donors through the Southwest Transplant Alliance. Diseased prostate specimens used in this study were obtained from patients undergoing transurethral resection of the prostate (TURP) or simple prostatectomy for symptomatic BPH at UT Southwestern Medical Center. Institutional Review Board approval was obtained for medical record review to retrospectively collect clinical and pathological data on each patient.

Similarity to Published OMIPs

None to date.

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