

Regulation of SLC1A4 and SLC1A5 in Prostate Cancer—Response

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In our study, we stated that SLC1A4 was a glutamine transporter. We acknowledge that this has never been directly shown. To that end, the first articles describing SLC1A4 (ASCT1), which was cloned from the brain, reported in 1993 that the protein was unable to transport glutamine in HeLa cells or *Xenopus* oocytes (1, 2). However, it should be noted that these studies did not agree on the transport of other amino acids such as cysteine. Furthermore, it was later determined that under conditions of stress such as low pH (similar to what is found in the tumor microenvironment that we tried to mimic in our study using serum starvation), the substrate specificity of SLC1A4 broadens to transport additional amino acids such as glutamate (3). In this regard, it has been noted that the substrate specificity of SLC1A4 is dependent on the cell type and tissue type (3). Given these studies, the data presented in our study demonstrating that knockdown of SLC1A4 decreases androgen-mediated glutamine uptake (Fig. 3B), and the published role of SLC1A4 in glutamine/glutamate cycling (4, 5), we think we cannot rule out the possibility that SLC1A4 could transport glutamine under some contexts. However, we must also accept that the androgen-mediated glutamine uptake we observed could be due to indirect effects whereby changes in the balance of intracellular amino acids transported by SLC1A4 may alter glutamine uptake or efflux through exchange transporters such as SLC1A5 (ASCT2) and LAT1. In addition, these changes could lead to the activation of a signaling molecule such as mTOR that could then increase the expression or activity of another glutamine transporter (e.g., SLC1A5 as we demonstrated). Certainly, the recent report by Dr. Ryan's group that was published after our study would argue against SLC1A4 acting as a direct transporter of glutamine (6). However, similar to prior studies, the initial evidence in Dr. Ryan's report that indicated SLC1A4 could not directly transport glutamine was first generated from experiments performed in *Xenopus* oocytes. These data were then elegantly validated using a prokaryotic cousin of the ASCTs, Glt_{ph} (~23% amino acid-sequence homology). But it is unclear whether these findings will accurately recapitulate what occurs in eukaryotic mammalian cancer cells under the harsh conditions of the tumor microenvironment. Clearly, new studies are warranted to determine whether SLC1A4 could transport glutamine directly under diverse conditions or whether this is only due to indirect effects.

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We also wish to acknowledge prior work from Wang and colleagues that demonstrated that androgens could increase the expression of *SLC1A4* and *SLC1A5* in LNCaP cells and their expression was increased in prostate cancer patient samples (7, 8). It is true that similar data for *SLC1A5* (alongside our mined *SLC1A4* clinical data) were presented in Dr. Holst's 2015 study (7), but it was unintentional as evidenced from our published work on unrelated topics (9) including work done by us well before any of these studies (10), demonstrating that this is a common format we use to present these types of clinical data. Importantly, while our study further validates the work of Wang and colleagues that defined the functional importance of SLC1A5/ASCT2 in prostate cancer (7, 8), there were some important differences. In addition to the new functional data presented on SLC1A4 and the mechanistic studies linking MYC and mTOR as additional upstream regulators of the transporters, all our studies were done under conditions to mimic the tumor microenvironment (serum starvation) compared with the prior studies done in the presence of serum. Furthermore, we extended our work to an additional AR⁺ cell line (VCaP) and focused on using lower concentrations of androgens (100 pmol/L) at which we and others observe peak proliferation of many androgen receptor (AR)⁺ cell lines including LNCaP and VCaP, as opposed to higher androgen concentrations (1–10 nmol/L) like the ones used in Wang and colleagues' 2013 study (8). At these higher concentrations, we and others find that androgen begins to block proliferation (a known biphasic effect of androgens) (11). Furthermore, in contrast to Wang and colleagues' 2013 findings, our data support an indirect (they reported a direct) AR regulation of *SLC1A4* and *SLC1A5* as evidenced by the late onset of mRNA expression and/or the ability of cycloheximide to block androgen-mediated induction under our conditions (serum starvation and 100 pmol/L androgen). In conjunction with one of our collaborators, Dr. Edwin Cheung, we mined his previous ChIP-Seq datasets (done in charcoal-stripped serum) and only detected significant androgen-induced AR binding in VCaP cells compared with LNCaP and C4-2 cells. Regardless, our studies collectively converge to highlight the importance of glutamine uptake and metabolism in prostate cancer and suggest that targeting the transport of this amino acid may have value in the treatment of the disease.

Disclosure of Potential Conflicts of Interest

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