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SHORT COMMUNICATION

Cell-to-Cell Communication in Prostate Differentiation and Cancer

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Introduction

We are taught that cancer is caused by accumulation of DNA mutations that activate oncogenes and inactivate suppressor genes. This mutation theory is, in several ways, quite unsatisfactory. First, mutation is a very rare event yet 13/100 men are diagnosed with prostate cancer in America each year, with higher incidence for Black men than White [1]. Second, for organs in the same anatomical area, cancer strikes the prostate the highest, the bladder lower, the seminal vesicles almost never. Third, why is the incidence higher in certain geographical regions than others? These imply that mutation, which should be non-selective, is anything but. One celebrated finding is the prostate cancer-specific TMPRSS2-ERG gene fusion [2]. However, this event shows a variable frequency in different world populations. Its only utility is in early detection for men who have this genomic alteration. Overall, a majority of cases are sporadic than familial. A small number of unfortunate men inherited mutated genes that makes them predisposed to cancer. Much effort has been spent in scanning the genome for disease-associated nucleotide changes [3]. Methods have been developed to knock out or knock in cancer-relevant gene candidates in mouse models to show that they are responsible [4]. Accordingly, cancer and progression to lethality is irreversible and incurable. We present a different take on how cancer develops and becomes lethal due rather to abnormal communication between cell types. Our study approach is to isolate by flow cytometry live cell populations from tumor and benign tissues of the prostate (and bladder for comparative analyses), determine their individual transcriptomes, and combine various cell populations in co-culture to observe interaction through secreted factors with or without cell contact. The experimental details can be found in our published articles in the reference list.

CD immunostaining of organ component cell types

In many aspects, prostate is an ideal human organ for research on intercellular signaling. The gland is a relatively simple organ composed of only three major cell types. Due to the high incidence of prostate cancer and open surgeries for its treatment one could have a reliable source of prostatic tissue, normal/benign and cancer, with informed patient consent for experimentation in cellular and molecular biology. Also available are metastases harvested from donor autopsies and their corresponding

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xenograft lines (e.g., the UW LuCaP family). For multicellular organs like the prostate and bladder, cell-to-cell communication via hormone molecules and heterotypic cell contact maintains proper differentiation of the component cell types. During embryogenesis, mesenchymal cells in the urogenital sinus instruct stem/progenitor cells to differentiate into functional mature epithelial or urothelial cells as appropriate [5,6]. Diseases such as neoplasia, hyperplasia, hypoplasia or dysplasia may develop if this communication is missing. It means that the instruction for terminal differentiation is no longer on hand leading to immature and not fully functional cells. We used immunostaining of Cluster Designation (CD) antibodies against cell surface antigens to tag the various cell types [7,8]. In the adult prostate, the stromal compartment contains CD49a+ smooth muscle cells (designated NPstrom – normal prostate stromal cells for convenience) and the epithelial compartment contains CD26+ luminal and CD104+ basal cells (plus a small number of neuroendocrine and possible organ progenitor cells) [9,10]. Other identifiable cell types include infiltrating CD45+ white blood cells, CD31+ endothelial cells of blood vessels, CD56+ nerve fiber cells. In adult bladder, the prostate stromal equivalent are CD13+ cells localized in the proximal lamina propria (NBstrom – normal bladder stromal cells) next to the urothelium of CD9+ urothelial and CD104+ basal cells (and progenitor cells) [11].

Principal components analysis plot of cell type-specific transcriptomes

As a means to study differentiation, we generated a 3D so-called Principal Components Analysis (PCA) plot from cell transcriptomes. Flow cytometry was used to purify the cell populations of CD49a+ stromal, CD26+ luminal, CD104+ basal, and CD31+ endothelial from the prostate [9,12], CD13+ stromal, CD9+ urothelial, and CD104+ basal from the bladder [11] for transcriptomics by DNA microarrays. For stem cells, we determined the transcriptomes of cultured Embryonic Stem (ES), Embryonal Carcinoma (EC), and induced Pluripotent (iPS) [13] cells. In this plot, the separation measured by a Δ value between any two transcriptome datapoints representing cell types indicates their degree of relatedness, the smaller the Δ the more related [12]. The stem cell types occupy a near center locale whereas the differentiated cell types are located toward the periphery with large Δ between them as well as between them individually and stem cells (Figure 1A). When the prostate and bladder PCA plots are displayed together, we can see the transcriptome (i.e., gene expression) difference between CD49a+ NPstrom and CD13+ NBstrom, between CD26+ prostate luminal and CD9+ bladder urothelial, and between their respective CD104+ basal (Figure 1B). The practical utility of this analysis tool will be demonstrated below. The main drawback is the requirement of a single platform to determine gene expression of all cell types. Previously, we showed that transcriptomes determined from laser-capture microdissected cell populations were not useful in generating such plots [14].

Stromal cell induction of stem cells

To demonstrate the functional property of stromal cells, we employed a co-culture of isolated stromal cells and stem cells represented by the EC cell line NCCIT, which has a gene signature similar to that of ES cells differing in only a small number of genes. The co-culture involved either growing the two cell types separated by a semi-permeable membrane barrier or one type with conditioned media of the other [12]. Interaction was monitored by cell/colony appearance and gene expression changes over a period of 7d. NCCIT cells in media of NPstrom or NBstrom became stromal-like by gene expression, best illustrated by down-regulation of the four stem cell Transcription Factors LIN28A, NANOG, POU5F1, SOX2 (scTF) and concomitant up-regulation of β2-microglobulin; (B2M). The PCA plot provides a visualization of the transcriptome change from 0h to 7d with the treated NCCIT datapoints "migrating" toward that of cultured stromal cells (Figure 1C). Note separation of the datapoints of cultured and flow-sorted Stromal (S), which is due mainly to genes activated in cell proliferation [15]. B2M expression is 10-fold less in stem and stem-like cancer cells than differentiated cells based on its DNA microarray signal intensity levels, which were verified by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) [16]. Robust colony growth typical of EC cells was greatly diminished with reduced cell density shown by gaps among colonies. Genes were induced differentially by NPstrom *vs.* NBstrom highlighting the ability of stem cells to respond to different sources of signaling or differentiation instructions [12].

What are the organ-specific (i.e., prostate *vs.* bladder) diffusible stromal factors present in the media? To answer, we carried out a comparative transcriptomic analysis between NPstrom and NBstrom for expressed genes encoding secreted

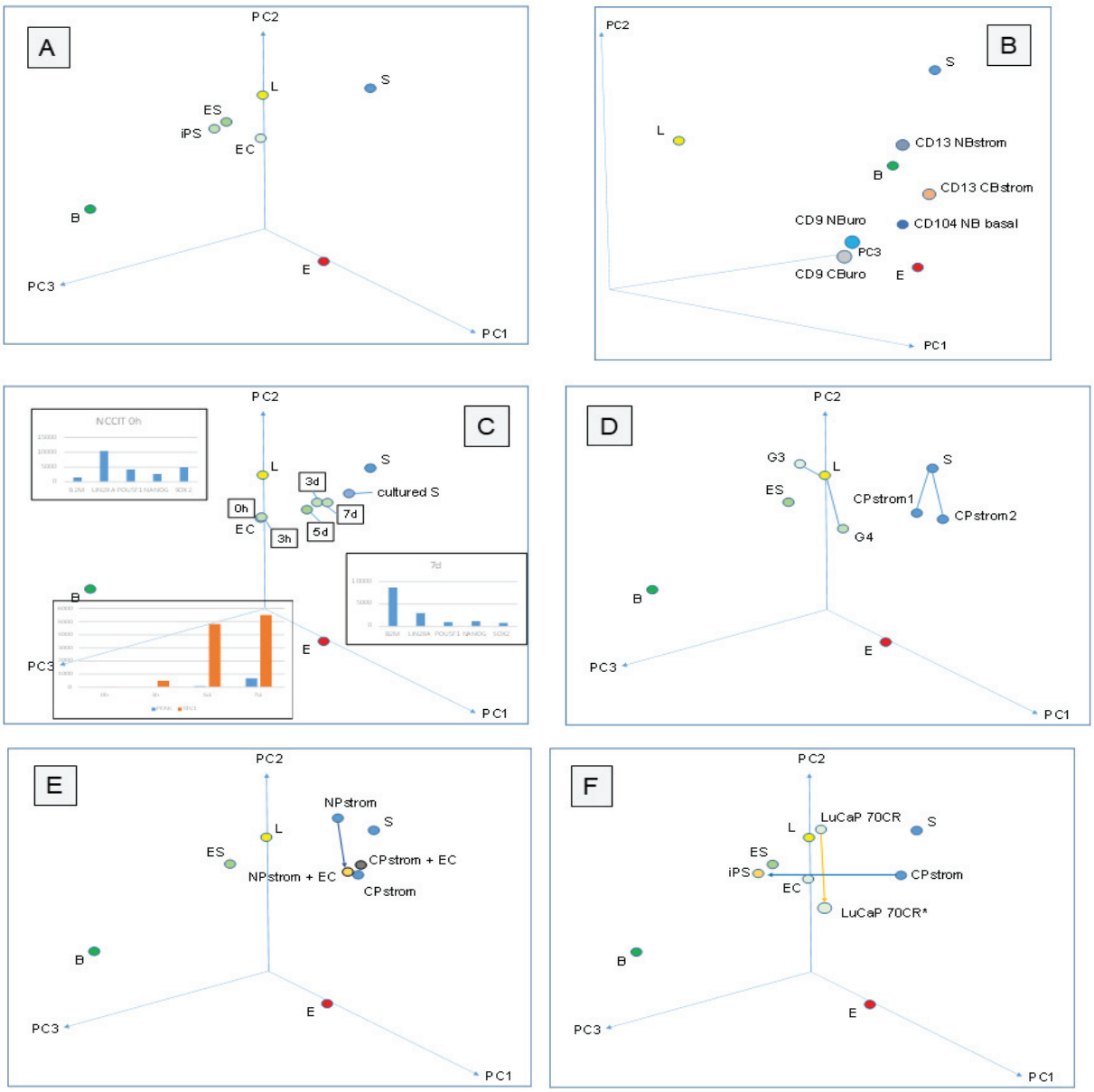

Figure 1 **PCA plot.**

These schematics are adapted from published data to show the following.

A. The prostate PCA is generated from the transcriptomes of luminal (L), stromal (S), basal (B) and endothelial (E) cells plus those of ES, EC and iPS cells.

B. Bladder cell-type transcriptomes are displayed in the prostate PCA space to show that bladder cell types – NBstrom, CBstrom, NBuro, CBuro, NBbasal – are distinct from the corresponding prostate ones. The 3D display is rotated for a different perspective of the datapoints than that in panel A.

C. Transcriptomes of induced NCCIT cells at various time points are visualized with respect to those of cultured and sorted (S) stromal cells. The expression levels of scTF, B2M at the beginning of co-culture (0h) and at 7d are shown in histogram format of DNA microarray signal intensity values (*y*-axis). The induction of STC1 and PENK in NCCIT by NPstrom in the time course is shown in the bottom left.

D. The gene expression difference between sorted NPstrom (S) and CPstrom, between sorted luminal (L) and cancers (G3, G4) are shown. The separation between L and G3 is smaller than that between S and CPstrom indicating more expression changes in CPstrom than G3 cancer from their normal counterpart.

E. The conversion of NPstrom by diffusible NCCIT factors in culture to CPstrom-like can be seen by the placement of the datapoints (mRNA) – NPstrom+NCCIT, CPstrom+NCCIT, CPstrom. NCCIT showed minimal effect on the transcriptome of CPstrom (CPstrom+NCCIT).

F. The changes in transcriptome between LuCaP 70CR and LuCaP 70CR*, between CPstrom and its iPS are compared. The respective Δ values are equally large.

proteins [17]. The bladder stromal cells are found in a 10-20-cell thick layer immunostained by CD13 in the lamina propria [11]. Prostate stromal cells are negative for CD13, although a number of other CD antigens are shared by both stromal cell types. We termed the CD13+ layer superficial lamina propria, which is also found in the mouse bladder [17]. The top identified genes for NPstrom were those that encode Proenkephalin (PENK), stanniocalcins STC1 and STC2. When these genes were queried from the transcriptome datasets of treated NCCIT cells, STC1 was detected early at 3h of co-culture, and its expression rose sharply over time, while PENK was detected later at 5d and, more important, not induced by NBstrom showing the specificity of stromal influence [12]. STC1 and STC2 were induced quantitatively different with higher STC1/lower STC2 by NPstrom *vs.* lower STC1/higher STC2 by NBstrom reflecting their expression patterns in CD49a+ NPstrom and CD13+ NBstrom [17].

Absent stromal PENK signaling in prostate cancer

PENK expression in the prostate was validated by immunohistochemistry using a polyclonal antibody raised against a selected peptide sequence. In addition to stromal staining, smooth muscle cells of large blood vessels and the bladder muscularis were positive [17]. Staining was absent in tumors, specifically the cancer-associated stroma. RT-PCR showed PENK expression minimal or undetectable in prostate tumors of different Gleason scores (3+3, 3+4, 4+5) as well as bone and liver metastases. Dataset query showed "present" only in the transcriptome of CD49a+ prostate stromal cells [17]. Cancer-associated stromal cells (CPstrom) are strongly positive for CD90 [18]. About a 20-cell width of CPstrom separates cancer epithelial cells from PENK-positive NPstrom of adjacent benign areas. Secreted PENK signaling is likely effective at a short distance, and could not affect cancer cells within tumor foci. Although lacking PENK, CD90-sorted CPstrom could still induce NCCIT to down-regulate the four scTF and up-regulate B2M indicating factors other than PENK could induce differentiation since Gleason pattern 3 (G3) tumors still possess a glandular histology [19,20]. Candidates include the STC proteins (see below), which, like PENK, have been reported to be involved in early development [21,22]. STC1 is expressed by both stromal and epithelial cells in NP and CP, which was verified by RT-PCR analysis and dataset query [17,20]. Its expression is decreased in cancer cells of advanced tumors and cancer cell lines [20]. As shown above, its induction in NCCIT predates that of PENK.

Difference between NPstrom and CPstrom

We showed by transcriptomics of sorted CD90+ CPstrom and CD49a+ NPstrom that the difference in gene expression was even more than that between luminal and G3 cancer [23,24] (Figure 1D). What is the nature of CPstrom? The interaction between co-cultured stromal cells and NCCIT was found bidirectional. Secreted stem factors from EC cells could convert co-cultured NPstrom into CPstrom-like based on transcriptomics [25] (Figure 1E). Cultured stromal fibroblastic cells initiated from different donor tissue specimens displayed a consistent gene expression profile with a correlation efficiency R = 0.99 [26]. Genes needed for cell division were activated in culture, but cell division was essential to produce changes in response to signaling. Co-culture of NCCIT and stromal cells lasted 3d. No gross morphology differences between NPstrom, CPstrom, or after co-culture with NCCIT were observed. However, co-cultured NPstrom were found to express qualitatively and quantitatively microRNA (miRNA) and mRNA similar to CPstrom. In contrast, no significant changes were found in co-cultured CPstrom. Examples of miRNA with increased expression include let-7f, miR-29b, miR-23a, miR-21. In particular, miR-21 is associated with cellular dedifferentiation [27]. Examples of mRNA increases include CD90, MiRN21, HGF, SFRP1, BGN and decreases include IGFBP5, HSD11B1. MiRN21 is the poly-adenylated, capped transcript to be processed to miR-21. The increase in CD90 corresponds to its stronger immunostaining of CPstrom. HGF (hepatocyte growth factor) is a known signaling molecule in stromal-epithelial interaction with high expression in the undifferentiated mesenchyme at embryogenesis and less in the adult [28]. Increased miR-21 and HGF is indicative that CPstrom represents a less differentiated state of NPstrom. Of note, tissue inhibitor of metallopeptidase TIMP3 was decreased and matrix metalloproteinase MMP1 was increased in CPstrom, which would affect integrity of the extracellular matrix allowing cancer cells to spread beyond the tumor foci [19]. These results suggest that unlike NPstrom, CPstrom is functionally defective in induction by not producing a key signaling factor PENK, which could then lead to abnormal epithelial cell differentiation, and perhaps cancer development [29].

Prostate cancer cell types

Prostate cancer shows loss of differentiation from low to higher Gleason, from tumors with glandular histology to tumors without, from adenocarcinoma to non-adenocarcinoma and small cell carcinoma. Based on transcriptome and placement in the PCA plot, prostate cancer cells can be either like luminal cells (differing in the expression of a few hundred genes [24]) or less like luminal and more like stem cells [30]. The luminal-like cancer cells include G3 cancer, LNCaP, C4-2, LuCaP adenocarcinoma cell lines. The stem-like cancer cells include Gleason 4 (G4) cancer, CL1, PC3, DU145, LuCaP non-adenocarcinoma and small cell carcinoma cell lines.

We showed a lineage relationship between these two groupings by using scTF vectors to transfect and reprogram five adenocarcinoma LuCaP lines. These were adapted to grow with Mouse Embryonic Fibroblasts (MEF) [31]. No significant expression changes were reported for the xenograft LuCaP 147 cells cultured *in vitro* as spheroids [32]. Reprogramming is an experimental process whereby differentiated cells are converted to iPS cells. The reprogrammed LuCaP cells became small cell carcinoma-like and stem-like in three weeks. The Δ between, for example, LuCaP 70CR (CR=Castration Resistant) and reprogrammed LuCaP 70CR* (* to indicate scTF-transfected) was similar to that between CPstrom and its iPS (Figure 1F). All five reprogrammed LuCaP derivatives appeared dark compared to their untransfected or mock transfected parentals, and were relatively smaller in size. The colony morphology was unlike that of cultured ES cells, and the dependence on MEF remained. RT-PCR showed that adenocarcinoma line LuCaP 23.12 was POU5F1+B2M^{hi} while small cell carcinoma LuCaP 145.1 was POU5F1+LIN28A+SOX2+NANOG+B2M^{lo} [31]. Thus, luminal-like cancer cells can be phenotyped as scTF-B2M^{hi} with respect to all four scTF *vs.* scTF+B2M^{lo} for stem-like cancer cells [16]. Loss of prostate cancer differentiation could be attributed to the activation of scTF as in cellular reprogramming.

PENK-induced differentiation of cancer cells

Could stromal factors such as PENK induce stem-like prostate cancer cells to undergo differentiation as in NPstrom induction of germ cell tumor-derived EC cells? We cloned PENK to transfect scTF+B2M^{lo} LuCaP 145.1. The cancer cells were adapted to grow *in vitro* with MEF after tissue digestion of a freshly resected xenograft [20]. Of note, LuCaP 145.1 cells have a lighter density (ρ = 1.035) than adenocarcinoma cells (ρ = 1.07) on banding in a discontinuous density gradient [16], another indication of its non-epithelial-like characteristics. At 3d post-transfection (when MEF were killed by the drug selection for transfected cells) with autocrine PENK production, down-regulation of scTF and up-regulation of B2M in transfected LuCaP 145.1 was found. The change from scTF+B2M^{lo} to scTF^{lo}/-B2M^{hi} was indicative of the cancer cells undergoing differentiation. The simultaneous changes in scTF and B2M were consistent with the results obtained in stromal induction of NCCIT. The decrease in POU5F1 was not as pronounced since non-stem-like LuCaP lines (LuCaP 23.12 and others) express this factor [31]. Control vector transfection produced no such result.

Next, is PENK capable of undoing cancer cell re-programming? We reprogrammed scTF-B2M^{hi} adenocarcinoma LNCaP by scTF vectors to scTF+B2M^{lo} LNCaP*. The obtained LNCaP* cells were cloned, and one clone (#2) was transfected by PENK. The resultant cells regained scTF-B2M^{hi} [20]. Individual LNCaP cells appeared with a "bright halo", irregular in cell shape with a tendency to cluster (under the culture condition used). LNCaP* cells appeared darker, more regular in shape. This appearance was similar to that of all the reprogrammed LuCaP* [31]. Individual cells grew in a loose formation not in contact with each other. LNCaP*/PENK appeared to regain the "bright halo" but the cell shape was distinct from that of LNCaP but similar to that of LNCaP transfected by PENK. Both grew in clusters (Figure 2, top panels). Transcriptomics showed multiple gene expression changes upon PENK transfection. One could say that luminal cells are held together by molecular tight junctions to restrict backflow of lumenal secretion, while cancer cells, especially of small cell carcinoma, being non-epithelial, do not form such tight junctions. The reprogramming reversal is accompanied by changes in cell appearance and colony morphology. In this sequence, cell appearance changed from LNCaP to stem-like LNCaP*, then to LNCaP*/PENK. STC1 could also produce the same effect as shown by LNCaP*/STC1 *vs.* LNCaP/STC1 (Figure 2, bottom panels). The oligonucleotides used for cloning the 760 bp STC1 cDNA were 5' primer cagggcccgatatcGAAACTTCTCAGAGAATGCTCCAAAACTCAG and 3' primer gctgaggatccTTATGCACTCTCATGGGATGTGCGTTTG. These experiments demonstrated that cancer cells, LuCaP 145.1 and LNCaP, could respond to activation

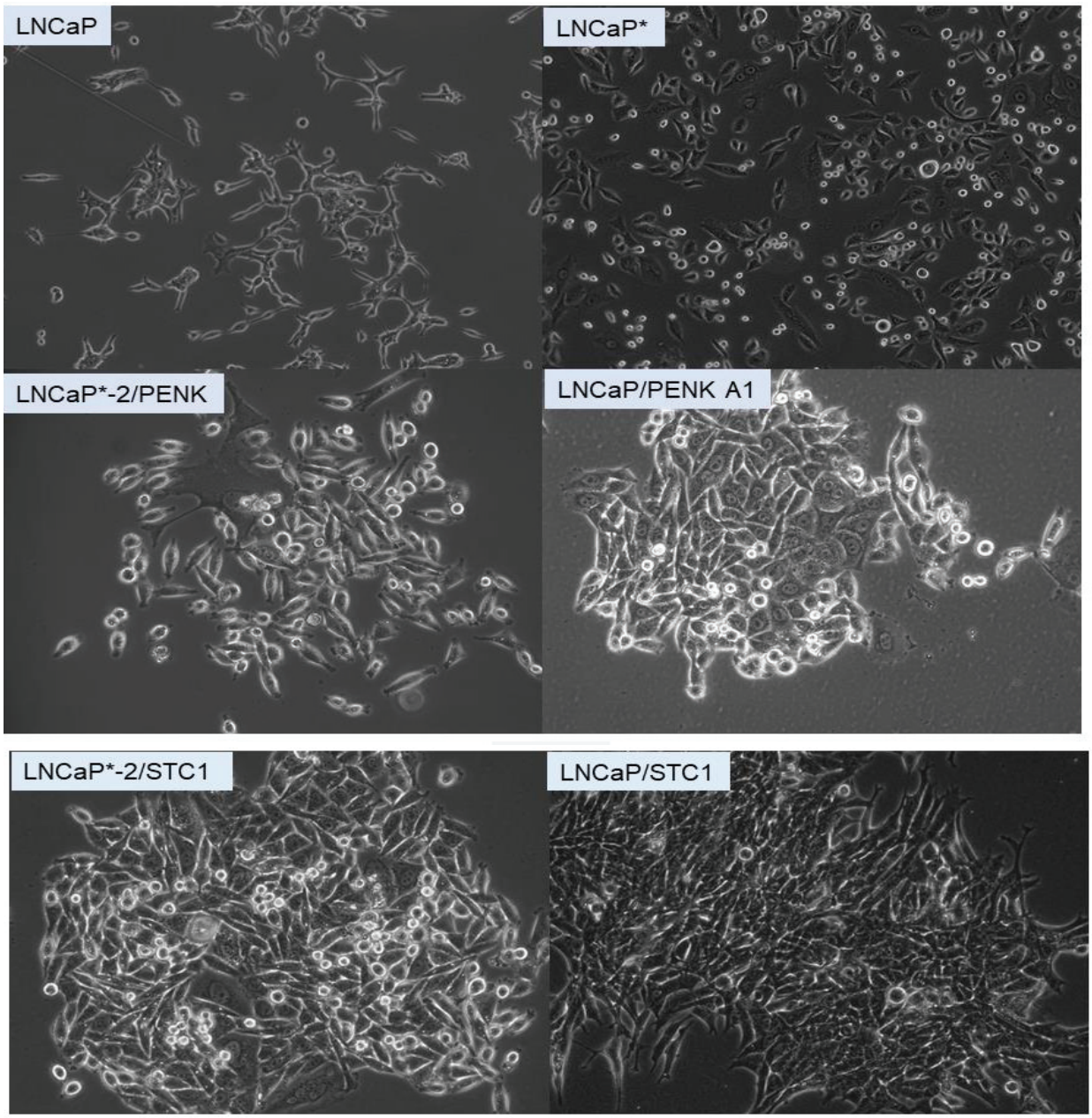

Figure 2 Cancer cell reprogramming and differentiation.

A. Top panels: LNCaP cells were reprogrammed by forced expression of scTF, and then induced to differentiate by forced expression of PENK. The colony morphology of the different lineage-related LNCaP cell types are visibly unique with those of LNCaP*/PENK and LNCaP/PENK (clone A1) appearing similar.

B. Bottom panels: STC1 could also produce morphology change in LNCaP* transfected by STC1 to appear similar to LNCaP transfected by STC1.

of scTF and to differentiation induction of PENK and STC1 despite containing multiple mutations in their genome and being aneuploid. It would then be expected not to pose a problem in the future application of PENK differentiation therapy to treat stem-like solid tumors. The reversal of reprogramming by PENK can explain our previous reported failure to reprogram PENK-positive NPstrom *vs.* PENK-negative CPstrom [13]. In principle, loss of cancer differentiation could be reversed or even prevented. With further research, it is possible that PENK plus others such as STC1 could induce cancer cells to a normal or pseudo-normal state (as shown by NPstrom induction of NCCIT). One prediction is that supplying key stromal factors miss-

ing in tumors (e.g., STC1 first followed by PENK as detected in NPstrom induction of NCCIT) could make cancer cells differentiate terminally to luminal-like cells. Differentiation therapy has been shown effective in treating certain leukemia where maturation of functional cells was promoted by identified chemical compounds [33]. As a side note, the down-regulation of B2M in stem-like cancer cells could undermine full expression of HLA-1 antigens (B2M being a subunit of the complex), which mediate cytotoxic T cell recognition of tumor cells. It is likely a mechanism behind the failure of immune checkpoint inhibition treatment of small cell lung cancer [34] if they are also scTF+B2M^{lo}.

Effect of cancer-associated antigen AGR2 on stromal cells

One might ask what happened to NP stromal cells where tumor emerged. Anterior Gradient 2 (AGR2) is known as an adenocarcinoma antigen due to its high expression in many solid tumors [35]. Cancer cells produce the extracellular form, eAGR2, where it is localized on the cell surface and secreted [35,36]. The intracellular form, iAGR2, is expressed by normal cells. In prostate cancer, AGR2-positive tumors are associated with better survival, even for high-stage diseases [37]. AGR2 expression is 10-fold higher in G3 cancer cells than that of G4 [24,38,39], suggesting its association with cancer differentiation. In local metastases, AGR2 is low or negative; another molecule, CD10, is a candidate responsible for extracapsular escape [40]. However, distant metastases contain cancer cells with high AGR2 (and low CD10) expression [37]. We showed that cancer-secreted AGR2 could induce Programmed Cell Death (PCD) of stromal cells characterized by cellular blebs, shrinkage, DNA fragmentation without RNA degradation as seen when stromal cells were UV-irradiated or treated by a pro-apoptotic drug staurosporine [41]. Necrotic stromal cells after electroporation showed both DNA and RNA degradation. In these experiments, low-passage stromal cells were cultured in the presence of AGR2. The source of AGR2 were tissue digestion media of adenocarcinoma LuCaP tumors (e.g., that of LuCaP 70CR containing >100 pg/ml AGR2), AGR2-positive tumor specimen (10-076CP), AGR2-positive bone metastasis. The control included digestion media of LuCaP 145.1 (containing <2 pg/ml AGR2), the corresponding AGR2-negative benign specimen (10-076NP). After 24h, no viable stromal cells were seen in AGR2-containing media whereas in control they remained healthy. Addition of the AGR2 antibody, P3A5 [42], to the media prevented PCD. Transcriptomics found down-regulation of spermidine/spermine N^1-acetyltransferase (SAT1) among the <30 (out of 54,000 represented by probesets) differentially expressed genes. SAT1 maintains intracellular polyamine levels; abnormal levels of which have an adverse effect through the induction of PCD [43]. SAT1 down-regulation was found in UV-irradiated stromal cells as well. Also identified was down-regulation of prothymosin-like α, which has an anti-apoptotic function. Circulating AGR2 in cancer patients could theoretically eliminate susceptible cells to allow metastatic cancer cells to invade and colonize other organs such as bone marrow, liver and lung [36]. Cells resistant to the effect of eAGR2 would not allow metastatic cancer cells to take root and expand, perhaps explaining preferential sites for prostate cancer metastasis. Inhibition of AGR2 by neutralizing antibodies could prevent tumor spread by targeting circulating surface eAGR2+ cancer cells and negating the deleterious effect of secreted AGR2 [44]. eAGR2 is a unique tumor-associated antigen in that normal cells only express iAGR2.

Summary

Defects in stromal cell signaling could contribute to cancer development. The term "reactive stroma" is used to describe changes found in the stromal compartment [45]. It implies that stromal cells react to the presence of cancer cells. These altered cells in turn promote cancer progression. Rather, it is the less differentiated state of CPstrom that cannot induce full differentiation of epithelial cells. Prostate cancer cells once exited the glandular capsule are no longer in contact with CPstrom, and yet they can still progress to lethality. Conversion from adenocarcinoma to small cell carcinoma occurs after androgen deprivation therapy as evidenced by both types containing TMPRSS2-ERG in the same patients for those positive for this biomarker [46]. Caner epithelial cells expression of eAGR2 further depletes functional stromal cells through PCD. Cancer expression of CD10 allows capsular escape. Increased expression of AGR2 again in the escaped cells allows spread to distant organs. Outside the prostate in metastases, cancer cells seem to become independent of stromal cells as evidenced by the adenocarcinoma histology and PSA secretion. PSA expression in the prostate by luminal cells is controlled through contact with stromal cells [47]. Cancer progression to lethality in later stages of the disease may arise in response to outside factors such as deprivation of androgen [46]. Figure 3 shows the different prostate cancer cell types in the disease course. Treatments need to be tailored to the different types.

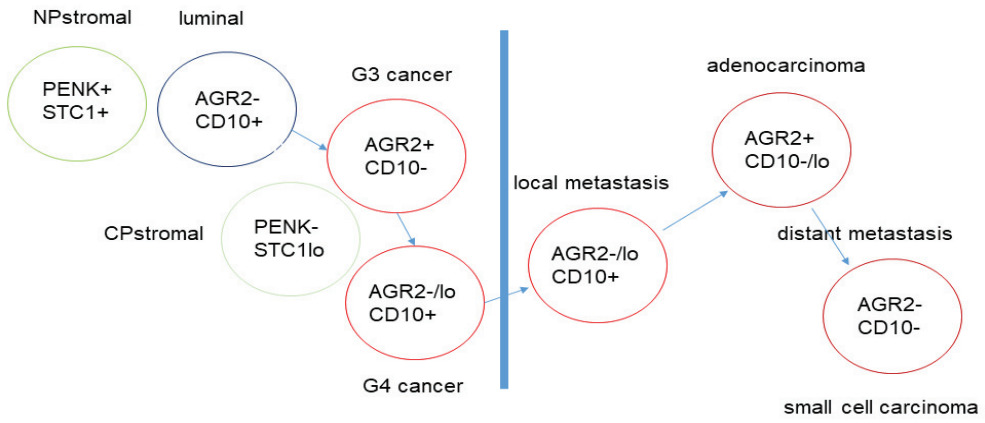

Figure 3 Lineage of prostate cancer cell types.

This schematic shows the evolution of prostate cancer cells at the primary site to local and distant metastases. The individual cell types are marked by key cell markers. The vertical bar represents the prostatic capsule. Expression of AGR2 and CD10 is variable among the different types

Anti-AGR2 immunotherapy could be effective against the eAGR2-positive (iAGR2-positive normal cells are immune) [48], while differentiation agents could be effective against the scTF-positive. PENK, STC1, and AGR2 are all involved in cell-cell interaction capable of producing phenotypic changes in responding cells. Our study shows that cancer progression (to a more stem-like state) can be reversed or prevented by stromal factors (as shown by NPstrom being resistant to reprogramming). Nuclear transcription factors, generally thought undruggable, can in fact be targeted by hormone molecules like PENK and STC1.

Limitations of all research employing human cells include ready availability of enough quantity (0.1 - 0.5 g) of tissue specimens for downstream processing. At present, availability is diminishing due to new techniques like pinpoint radiation and laparoscopy where targeted tissues are extensively damaged. Because of screening, higher-grade cancer are less frequently diagnosed. Optimally, one would like a transcriptome dataset of 10-20 sorted G3, G4 and Gleason 5 tumor cells. Fortunately, many more xenografts representative of the disease are being established [49]. There remains the somewhat tedious process of adapting them to *in vitro* growth where care is exercised to ensure mouse fibroblasts in the harvested tumors are completely removed otherwise these cells will overgrow. Stromal cells, NPstrom or CPstrom, once isolated (e.g., from excess biopsies) can be cultured for multiple passages, and stored frozen. Currently, lack of monoclonal antibodies to PENK, STC1 is hindering in their purification for adding directly to tumor cells *in vitro* and in mice.

Our research approach is applicable to other major organs. Tissue regeneration and renewal, as shown by NPstrom and NBstrom induction of NCCIT, require organ-specific instructive stromal factors, which are yet unidentified for many. We presented a CD signature of kidney component cell types (~30) [14], which can be utilized to isolate and study renal cell differentiation. Although tissue progenitor cells are postulated to be present, e.g., side population of the prostate [10], their scarcity presents a challenge in isolation. Nevertheless, patient-derived iPS cells can be used instead. Reprogramming is readily achieved with our constructed scTF plasmid vectors [16].

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