Clonal heterogeneity in patient-derived xenografts: The Neuroendocrine PDX model BL0479 contains stable clones with epithelial or mesenchymal characteristics and differential drug sensitivities

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Introduction

It is well established that cancers are heterogeneous diseases with biological, genetic, and histopathological differences among patients and within individual tumors. Consequently, it is not surprising that animal models based on established cell lines with their clonal nature and adaptations to defined media have limited preclinical value. Recent advances in the generation of patient-derived xenografts (PDX), where patient material is engrafted into immunosuppressed mice, suggests that this approach may provide a more representative surrogate for therapeutic development. In this study, a mixed cell culture derived from a neuroendocrine cancer PDX model was analyzed for cellular heterogeneity.

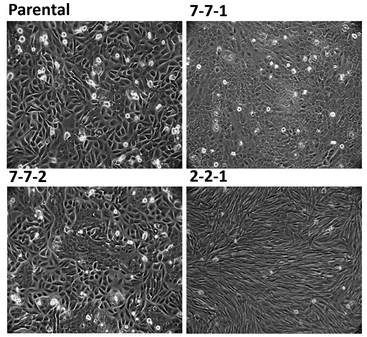


Figure 1: Phase contrast images [20x] of parental culture and clones – note morphology differential.

Gene	Chr	Pos	Ref	Alt	AA change
AKT2	19	40761140	T	C	N71S
KDR	4	55979558	C	T	V297I
MGMT	10	131506283	C	T	L115F
TP53	17	7577099	C	G	R280T

Table 1: Mutations conserved in parental BL0479 and all clones

Gene Symbol	DCT Values Fibroblasts	Clone 7-7-1	Clone 7-7-2	Clone 2-2-1
VIM	3.6	6.7	5.4	3.2
COL1A2	4.4	6.5	7.0	6.9
GREM1	4.7	8.5	8.8	8.3
KRT18	5.1	2.6	3.2	4.4
LOX	5.4	8.9	9.3	8.9
COL3A1	5.5	13.6	13.8	12.7
ACTA2	5.6	11.0	11.3	9.5
CD248	6.5	5.9	6.6	5.2
KRT8	6.7	4.3	4.2	4.2
CD44	7.3	4.1	5.2	4.5
CD24	8.8	5.5	5.9	6.2
KIT	9.1	9.3	9.1	8.1
VCAM-1	9.5	15.4	14.8	12.2
KRT7	9.6	2.9	3.1	4.4
KRT5	10.1	1.3	2.3	7.0
EPCAM	10.7	7.7	8.8	14.1
KRT14	11.0	11.5	13.2	11.3
KRT19	11.0	4.5	4.8	5.9
PECAM1	11.3	13.8	12.8	9.5
KRT15	12.1	9.5	10.1	10.8
CDH1	13.2	9.0	9.6	13.7

Figure 2: A qRT-PCR array designed to detect fibroblasts confirms that clone 2-2-1 is 1) not a fibroblast and 2) differs in expression from 7-7-1 and 7-7-2.

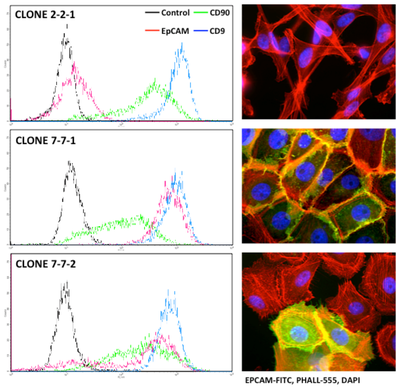


Figure 4: FACS [EPCAM. CD9, CD90] and ICC [EPCAM] analysis of clones

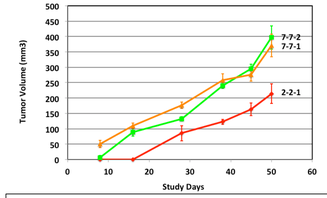


Figure 3: Growth of isolated clones *in vivo* – tumorigencity retained.

Fold Change vs. Parental ID	Clone 7-7-1	Clone 7-7-2	Clone 2-2-1
CAMK2N1	1.04	-1.80	-3.71
CDH1	-1.08	-1.88	-235.23
CDH2	-3.94	-2.92	-4.51
CLDN1	1.34	-1.16	-9.18
COL1A1	1.00	3.96	14.94
COL1A2	-1.35	1.29	7.69
COL5A2	1.05	4.19	11.09
DSC2	-1.60	-4.23	-36.14
DSG3	7.21	1.22	-2.19
DSP	-1.55	-2.68	-6.11
F11R	-1.91	-2.10	-3.42
FGFBP1	3.12	2.28	1.05
FN1	-2.32	-2.19	-4.59
GNG11	-2.54	10.51	13.96
IGFBP4	-1.09	1.64	9.86
ITGA5	-3.95	-4.50	-2.09
JAG1	-1.88	-3.42	-2.07
KRT13	22.70	20.95	-1.79
KRT14	-1.30	-2.67	-3.66
KRT15	11.72	6.32	-2.14
KRT17	1.38	-4.05	-29.35
MMP2	-1.18	2.08	24.11
OCLN	-1.92	-2.80	-3.31
PDGFRB	1.20	1.69	3.56
PTP4A1	-3.28	-2.74	-2.65
RGS2	2.26	8.67	4.80
SERPINE1	6.73	8.44	2.98
STEAP1	-5.83	-6.00	-4.32
TCF4	-4.41	-5.99	1.20
TFPI2	-7.93	2.11	2.32
TGFB2	-2.57	-2.97	-4.05
TIMP1	1.16	1.46	3.51
TWIST1	4.33	7.64	9.28
VCAN	-67.40	-62.95	-10.24
VIM	-1.71	3.47	10.75
WNT5A	-1.35	8.65	-1.17
ZEB1	-3.03	2.97	12.56

Figure 5: Differentials in EMT-related genes from Affy U133 Plus 2.0 microarray data of three clones.

Results and Discussion

Several clones were isolated from a PDX model of Neuroendocrine cancer BL0479 [Original sample from Jackson Laboratories].

- Clones differed morphologically – clone 2-2-1 had an elongated/spindle shape whereas 7-7-1 was cuboidal. Clone 7-7-2 and the parental appeared to contain both morphologies (Figure 1).
- All clones harbored the same NCI gene panel non-synonymous variants (Table 1).
- A custom qRT-PCR array confirmed that none of the clones had a fibroblast signature (Figure 2).
- All clones also retained *in vivo* tumorigenicity (Figure 3).
- Clones had an expression differential in terms of EPCAM and CD90 (FACS/ICC analysis – Figure 4). Clone 7-7-1 was EPCAM+++/CD90++ and Clone 2-2-1 was EPCAM-/CD90+++, whereas clone 7-7-2 had a mixed phenotype.
- Microarray analysis and western blotting confirmed that clone 2-2-1 had likely undergone epithelial to mesenchymal [EMT] transition (Figures 5 and 6).
- Compound testing *in vitro* revealed that clone 2-2-1 was more sensitive than the other clones against a panel of clinically relevant agents (trametinib, everolimus, temozolomide, ABT-888, carboplatin & MK 1775) (Figure 7) – A counterintuitive result given that EMT is regarded as conferring drug resistance.
- Combination studies *in vitro* revealed that all clones were highly sensitive [high degree of synergy] vs. a combination of temozolomide & ABT-888 (Figure 8). A plausible explanation for this sensitivity is the lack of O6-Methylguanine-DNA Methyltransferase (MGMT) expression across all clones.

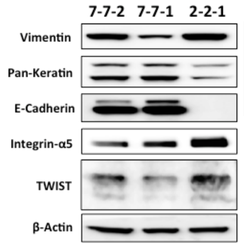


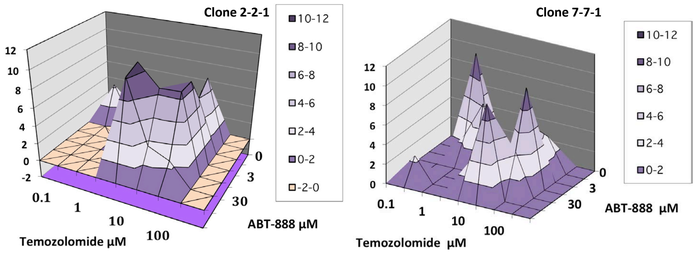
Figure 6: Western blot validation of select EMT transition markers in the three clones.

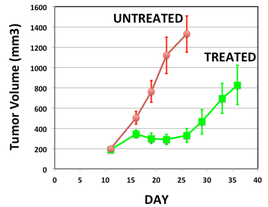
	IC50 µM Parental	Clone 7-7-1	Clone 7-7-2	Clone 2-2-1
Temozolamide	90	80	110	21
Everolimus	>1	>1	>1	2
MK1775	>1	2	0.17	0.04
Carboplatin	18	3.8	3.6	1.9
Trametinib	>1	>1	>1	>1
ABT-888	>100	110	180	50

Figure 7: IC50 values for single agent studies

	Combination Temozolamide + ABT-888 Synergy 99%	Antagonism 99%
Parental	40.37	0
Clone 7-7-1	57.1	0
Clone 7-7-2	117.91	0
Clone 2-2-1	74.45	-1.81

Figure 8: Temozolomide + ABT888 combination synergy studies showed high levels of synergy for this combo *in vitro* as shown in 3-D contour plots.





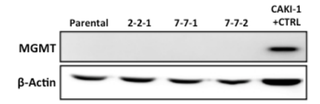


Figure 9: Temozolomide + ABT888 sensitivity is evident *in vivo* against parental cells [left] and all clones fail to express MGMT [top].

Conclusions

A central advantage of patient-derived xenografts (PDX) is their ability to recapitulate patient disease in terms of tumor cell heterogeneity. In this study, a PDX model of neuroendocrine cancer was investigated to determine the extent of any heterogeneity. Results demonstrated that two clonotypes predominated, one with epithelial characteristics and the other with a mesenchymal signature. The two forms also had markedly different responses to a panel of anti-cancer agents. These data boradly support the inference that PDX models are superior to classical models for *in vivo* studies.

REVISED FROM ORIGINAL: Model Name & diagnosis corrected