

Table 1. Summary of Melanoma Cases by Cluster Designation

Melanoma Primary Cluster

Case No.	Sex/Age	Biopsy Site	Passage No. (Biopsy)	p16 Mutation Status ^a	Invasive Ability ^b	Vasulogenic Mimicry ^c	Gel Contraction ^d	Cell Motility ^e	Scratch Wound (%) ^f
UACC-502	M/69	Cervical Node	3	Deleted	2.8± 0.1%	-	ND	ND	37
M92-001	F/43	Ankle	2	Deleted	3.0±0.5%	-	ND	76.80±(2.96)	22
A-375	F/54	Skin	ND	Mutation	2.8±0.2%	-	ND	67.80±(4.40)	26
M91-054*	M/45	Axill. Lymph Node	3	WT	*	*	*	ND	30
UACC-1256	F/67	Thigh Femoral Node	9	Deleted	ND	ND	ND	ND	ND
M93-007	F/43	Ankle	3	Deleted	2.6±0.1%	-	-	ND	12
UACC-091	M/52	Ukn	7	Deleted	2.1±0.2%	-	-	ND	11
UACC-1273	M/50	Axill. Lymph Node	16	Mutation	2.5±0.3%	-	-	ND	13
TD-1730	M/55	Thyroid Lobe	Biopsy	ND	ND	ND	ND	ND	ND
TD-1638	M/49	Paraspinous	Biopsy	ND	ND	ND	ND	ND	ND
TD-1720	M/29	Shoulder	Biopsy	ND	ND	ND	ND	ND	ND
TD-1348	M/44	Axill. Lymph Node	Biopsy	ND	ND	ND	ND	ND	ND
UACC-1022	F/53	Chest Wall	13	WT	2.9±0.1%	-	-	ND	63
TC-1376**	M/30	Distal Ilium	3	ND	ND	ND	ND	ND	21
TD-1376**	M/30	Distal Ilium	Biopsy	ND	ND	ND	ND	ND	ND
UACC-2534	M/68	Abdomen	7	Deleted	3.2±0.02%	-	ND	ND	7
UACC-383	M/69	Thigh Femoral Node	29	Deleted	2.3±0.2%	-	ND	70.40±(5.27)	35
UACC-457	F/Ukn	Ukn	19	WT	3.1±0.2%	-	ND	12.80±(0.05)	ND
UACC-3093	M/75	Axill. Lymph Node	4	WT	ND	ND	ND	40.30±(2.00)	24

Melanoma Non-Clustered

UACC-930	F/35	Sm. Bowel	4	WT	4.8±0.3%	+/-	-	ND	50
M93-047	F/75	Axill. Lymph Node	3	Mutation	10.7±0.3%	+	+	ND	75
UACC-2873	M/37	Axill. Lymph Node	5	ND	ND	ND	ND	ND	48
UACC-903	M/25	Back	14	Deleted	3.8±0.3%	+	-	ND	91
TC-FO27	M/30	Rt. Chest Wall	6	ND	ND	ND	ND	ND	91
UACC-1097	M/56	Rectus Muscle	6	Mutation	ND	ND	ND	ND	34
UACC-647	M/32	Axill. Node	14	WT	3.8±0.1%	+	+/-	ND	55
UACC-1012	M/54	Neck	3	ND	4.9±0.1%	ND	ND	122.00±(11.30)	54
UACC-827	F/32	Rt. Breast	16	Mutation	ND	ND	ND	ND	32
WM1791C	Unk	Ukn	52	ND	4.6±0.3%	+	ND	141.00±(11.40)	71
HA-A	F/Ukn	Ukn	19	ND	3.9±0.5%	+/-	ND	211.00±(12.40)	62
UACC-1529	M/48	Axill. Lymph Node	13	Mutation	4.2±0.5%	+	-	ND	ND

Uveal Melanoma Samples

OCM-1A	Ukn	Primary	25	ND	2.2 ± 0.1%	-	-	ND	ND
C918	F/60	Primary	15	ND	12.9± 0.3%	+	+	ND	ND
MUM-2C	M	Liver Metastasis	8	ND	2.0 ± 0.1%	-	-	ND	ND
MUM-2B	M	Liver Metastasis	8	ND	13.3 ± 0.6%	+	+	ND	ND

Control Samples

Nil.C(Fibroblast); UACC-3149(Ovarian Adenocarcinoma); MCF-10A(Breast Epithelium); CRL-1634(Fibroblast); SRS-3(Cell Culture Variant); SRS-5(Cell Culture Variant); RMS-13(Rhabdomyosarcoma)

LEGEND

ND = not determined; WT = wild type; Ukn = unknown

^a Mutation status of indicated samples for p16, with "Deleted" indicating homozygous loss of p16. Results was determined by direct sequencing of all exons from and p16. Supplemental information found at (<http://www.nhgri.nih.gov/Nature/>) provides the specific mutations in p16 for each sample tested. In addition all samples were directly sequenced for β-catenin coding sequence change. No example of any B-catenin mutation was observed.

^b (p=0.0055, t-test for two populations) Tumor cells (1 x 10⁵) were seeded onto collagen/laminin/gelatin-coated (Sigma) polycarbonate membranes containing 10-μm pores (Osmonics, Livermore, CA). After 24 hours of incubation at 37°C, the cells that invaded each membrane were collected, stained, and counted.

Percent invasion was corrected for proliferation and calculated as follows: $\frac{\text{total number of invading cells}}{\text{total number of cells seeded}} \times 100$ (see ref. 5)

^c tube forming ability at 5 days in a 3-D matrigel matrix (see ref. 5)

^d ability to contract floating collagen I gels at 5 days as compared to HT-1080 fibrosarcoma cell line (see ref.5)

^e Migration rates are expressed in (μm/24h) with rates below 100um/24hrs completely segregating the melanoma primary cluster from the non-clustered melanomas. Calculated values are the mean from eight measurements +/- s.d. (p = 0.0063, t-test for two populations)

^f ability to close in vitro scratch wound at 24 hours, photographs of wound were measured and percentage wound closure determined (see ref. 23) (p<0.00002, t-test for two populations)

*M91-054 was the only sample in the study which demonstrated a mixed phenotype in culture with both an epithelioid pop. and a more fibroblastic pop. Vasculogenic mimicry and gel contraction were observed only in the epithelioid population which was assayed for these properties. The scratch assay was done on both populations and resulted in 30% closure at 24 hours.

**TC-1376 mRNA was isolated after short term (3 passage) culture of the biopsy sample from patient TD-1376 allowing the direct comparison in this patient of the effects on short term culture on the expression profile.

^oUACC-647 cells formed extensive cord-like networks by 5 days.