Genomic Characterization of Immortalized NF1 Schwann Cells

Peggy Wallace[1], Hua Li[1], Alejandro Sanoja[1], Lung-Ji Chang[1], David Muir[2], Marigo Stathis[3], Jaishri Blakely[3], Justin Guinney[4], Sara Gosline[4].

1) University of Florida, Molecular Genetics and Microbiology. 2) University of Florida, Pediatrics.
3) Neurofibromatosis Therapeutic Acceleration Program, Johns Hopkins University. 4) Sage Bionetworks

Introduction

Schwann cell (SC) cultures from NF1 plexiform neurofibromas (pNF) typically are impure and do not divide more than 6-10 passages. To provide a more robust cell biology resource, we immortalized these two-hit (-/-) cells (and normal (wild type) and heterozygous SC (from non-tumor NF1 nerve) using retroviruses (originally) and lentiviruses carrying human telomerase catalytic subunit (TERT) and murine Cdk4 (Li et al., in revision). All resulting cell lines ("i") have divided to at least p50, and most have lost laminin and neuregulin dependence/preference. These cells were subjected to cell biological and genetic analyses, in comparison to the primary cultured cells. Representative data from some such studies are shown here, indicating validation and utlility of the lines.

Table 1. Demographics and *NF1* germline mutation for three pNF cell lines (all males). Somatic mutation for ipNF95.6 is R2237X, while the other two lines have large deletions.

Culture name	Age	Familial?	*NF1* germline mutation
ipNF05.5	32	no	c.3456_3457insA framshift
ipNF95.6	6	yes	c.2446 C>T R816X
ipNF95.11b	19	no	c.1756delACTA frameshift

Figure 1. Two-hit (-/-) immortalized SC may change morphology. S100B immunocytochemistry staining. Panel A shows primary pNF95.11b SC. B shows the resulting ipNF95.11b cell line from immortalization with retroviruses (line called "C/T"), and the cell line in panel C resulted from transduction with one retrovirally-delivered transgene and lentiviral delivery of the other gene (line called "C").

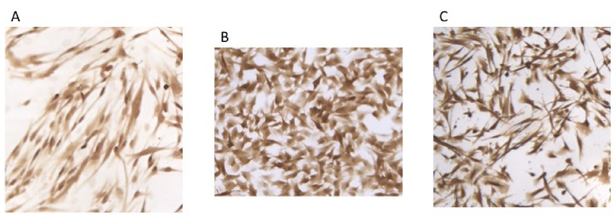
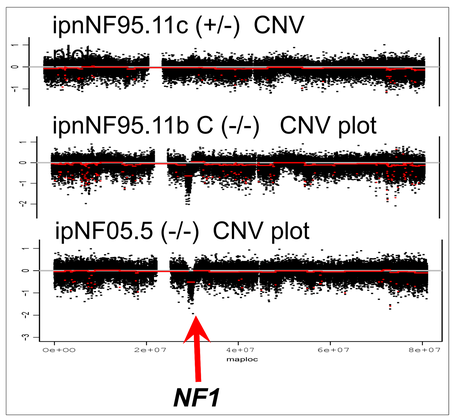


Table 2. STR sample data authenticates heterozygous &two-hit immortalized SC lines; genotypes match primary culture pNF95.11b.

STR marker	ipnNF95.11c (+/-)	ipNF95.11b C (-/-)	ipNF95.11b C/T (-.-)
D7S820	10,12	10,12	10,12
D16S539	13,14	13,14	13,14
D3S1358	17	17	17
PentaD	8,11	8,11	8,11

Figure 2. CNV analysis (top box) based on SNP array data shows nearly identical somatic deletion of the *NF1* region in two -/- cell lines, but not a heterogyzoug line, validated with PacI digest-based loss of heterozygosity analysis at rs964288 in the *NF1* gene (bottom panel).



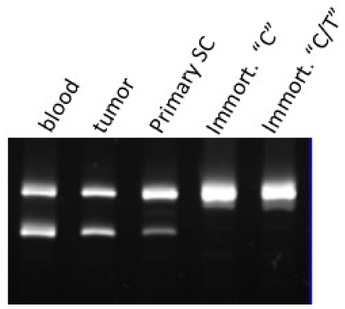
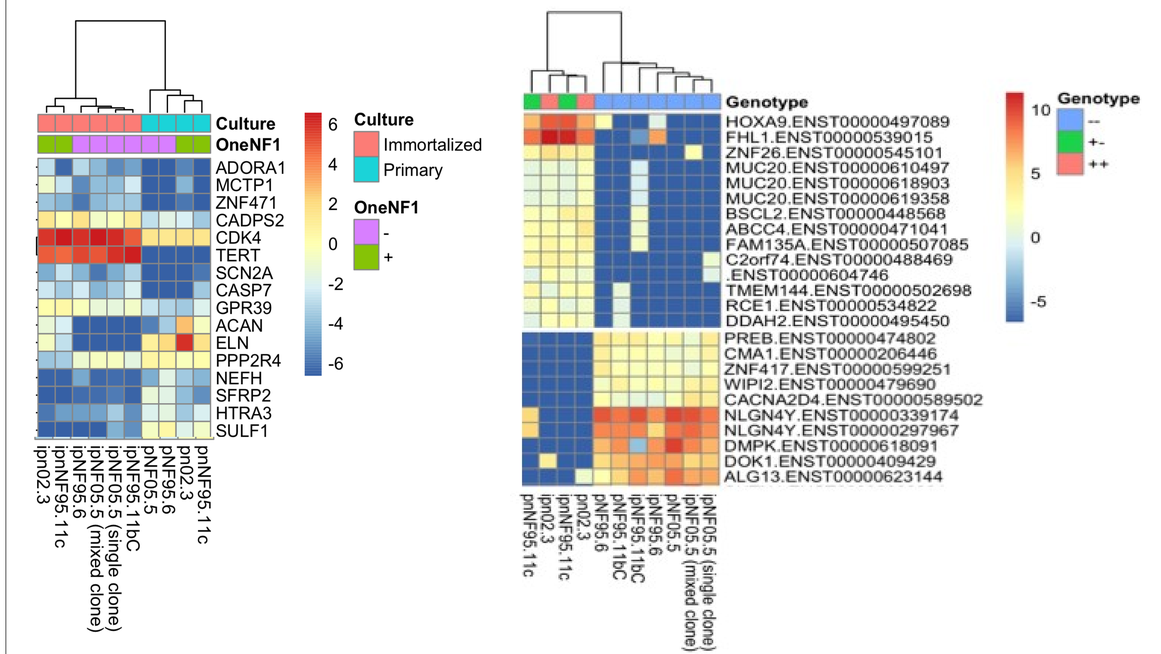


Table 3. Karyotypes of immortal SC lines, same patient in same color. All primary SC cultures were 46,XY. "clone mix" is a mix of 6 single cell clones at p16 (isolated at p11). Some cells gained abnormalities; ipNF05.5 has a clonal translocation predominating.

Culture name	SC (NF1 status)	karyotype (passage)
ipNF05.5	pNF (-/-)	46,XY,t(2;3)(q23;p26)[18]/46~47,sl,+9[cp2] (p41)
ipNF05.5 clone mix	6 (-/-) clones	46,XY,t(2;3)(q23;p26)[20] (p32)
ipNF95.11b C	pNF (-/-)	46,XY,ins(2)(q37.3p24p23)[cp11]/46,sl,del(21)(q22.3)[9] (p56)
ipNF95.11b C/T	pNF (-/-)	46,XY [20] (p45)
ipnNF95.11c	nerve (+/-)	46,XY [20] (p24)
ipNF95.6	pNF (-/-)	46,XY[19]/92,XXYY[1] (p53)

Figure 3 Left: RNA sequence data validates over-expression of hTERT and mCDK4 in immortalized cells; some genes differ in epxression between the primary and immortal lines. Right: some genes are expressed consistently among lines from each culture.



Discussion: The immortalized pNF cell lines appear pure for two-hit SC and can divide indefinitely. In some cases immortalization was accompanied by cytogenetic abnormalities, but these appear relatively stable. Gene expression profiles from transcriptome data show some consistency with primary cultures, and some differences (expected, given alteration in senescence programs). As expected, the heterozygous cell line's RNA profile is more similar to wild type SC than -/- SC. Single cell cloning is possible but may not provide any advantages, based on the data in this study. Exome data analyses are underway as well. These cells provide another tool in NF1 research, including in preclinical in vitro and in vivo studies (see Gosline poster, and Li et al., in revision, *Laboratory Investigation*).