High-Level Gene Amplification in Human Pluripotent Stem Cells

Erik McIntire, Kimberly Leonhard, Tenneille Ludwig, Benjamin Nisler, Anna Lisa Larson, Gustavo Velazquez, Erica Schutter, Brenna Anderson, Seth Taapken
WiCell, Madison, WI, USA

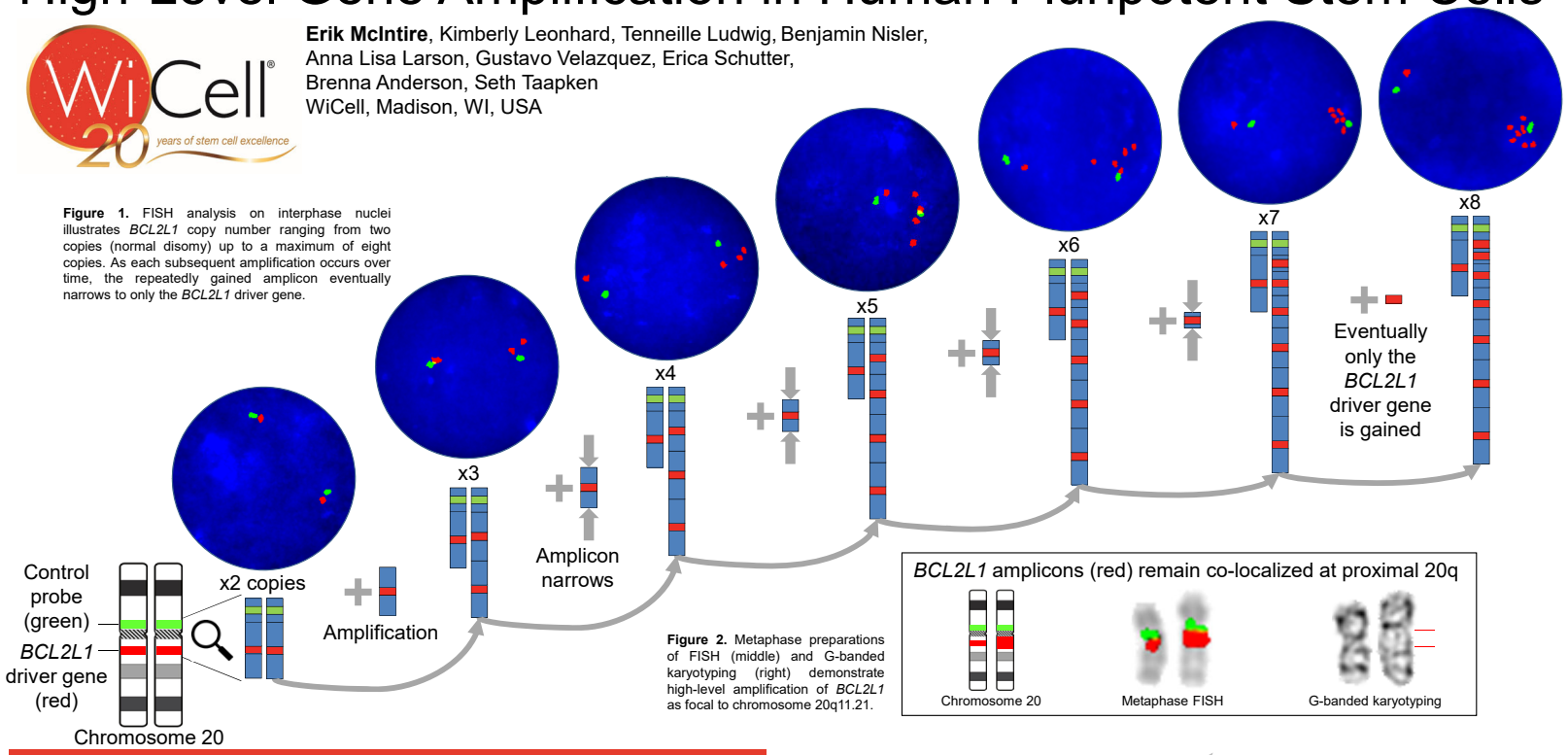

Figure 1. FISH analysis on interphase nuclei illustrates *BCL2L1* copy number ranging from two copies (normal disomy) up to a maximum of eight copies. As each subsequent amplification occurs over time, the repeatedly gained amplicon eventually narrows to only the *BCL2L1* driver gene.

Figure 2. Metaphase preparations of FISH (middle) and G-banded karyotyping (right) demonstrate high-level amplification of *BCL2L1* as focal to chromosome 20q11.21.

Introduction

Human pluripotent stem cells (hPSC) acquire recurrent genetic abnormalities during culture, which confer a selective growth advantage over wild-type cells. Recurrently acquired abnormalities in hPSC parallel those observed in certain cancers, and potentially affect the research fidelity and clinical safety of hPSC applications. Most recurrent abnormalities are whole-chromosome gains or duplications[1], which result in one additional copy of the gained region. High-level gene amplification (defined here as at least a four-fold increase, or ≥8 total copies) of a recurrently acquired abnormality is routinely observed in cancer[2] but unreported in hPSC. We characterized novel high-level amplification of the gene *BCL2L1* in stem cells by utilizing three different cytogenetic techniques. *BCL2L1* is the driver gene of the most commonly acquired recurrent abnormality in hPSC, gain of chromosome 20[3].

Materials and Methods

G-banded karyotyping was carried out using standard cytogenetic protocols modified for use with hPSC. Fluorescence *in situ* hybridization (FISH) probes for the *BCL2L1* gene region (Illumina, RP5-857M17) and a control probe (Empire Genomics, CHR20-10-GR) were hybridized according to the manufacturers' protocols. G-banded karyotyping and FISH preparations were digitally captured and analyzed with Applied Spectral Imaging equipment. Finally, chromosomal microarray (CMA) was performed using genomic DNA hybridized to the Illumina CytoSNP-850K BeadChip and analyzed using BlueFuse v4.4 and the GRCh37/hg19 human genome assembly.

Results

FISH analysis for *BCL2L1* copy number on interphase nuclei revealed subpopulations ranging from two copies (normal disomy) up to a maximum of eight copies (Figure 1). FISH and karyotype analysis of metaphase preparations (Figure 2) demonstrated the amplification to be focal, with all *BCL2L1* amplicons co-located to chromosome 20q11.21. Microarray results delineated a total amplified region of 888kb (genomic position chr20: 29,804,293-30,691,922) that contained a stepwise amplification pattern of increasing copy number gains (Figure 3). Stepwise amplification patterns typically result from constant selection pressure, in which the repeatedly gained amplicon narrows to only the driver gene (in this case, *BCL2L1*) at the region's apex. These patterns identify potential driver genes in cancer[2]; as *BCL2L1* was previously confirmed as a driver gene in hPSC through other methods[3], this finding demonstrates the same mapping utility in hPSC. Therefore, we applied this mapping strategy to another recurrent abnormality with a known minimal overlapping region: gain of chromosome 1q, refined to cytoband 1q32.1. We performed microarray testing on hPSC cultures with 1q32.1 gain; results identified the smallest measured size of the recurrent region at 1.9Mb and containing 39 genes (genomic position chr1: 202,780,530-204,633,340). This amplified 1q region appears to exhibit a nascent stepwise amplification pattern (Figure 4) though comparatively less defined. Based upon copy number and function, a credible driver gene candidate for 1q32.1 recurrent gain in hPSC is *MDM4*, which reportedly inhibits p53 activity[4]. The emergence of high-level gene amplification in hPSC is concerning as it is an additional parallel with cancer[1,2]. However, high-level amplification also provides an opportunity to map driver genes, thereby enabling targeted screening and providing insight into the functional consequences of recurrent abnormalities in hPSC.

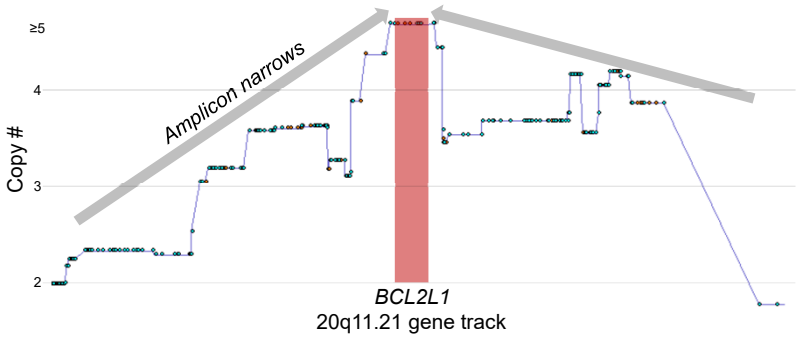

Figure 3. Microarray results of the amplified region on chromosome 20q11.21. Results illustrate a stepwise amplification pattern of increasing copy number gains across the region, with the *BCL2L1* driver gene at the apex.

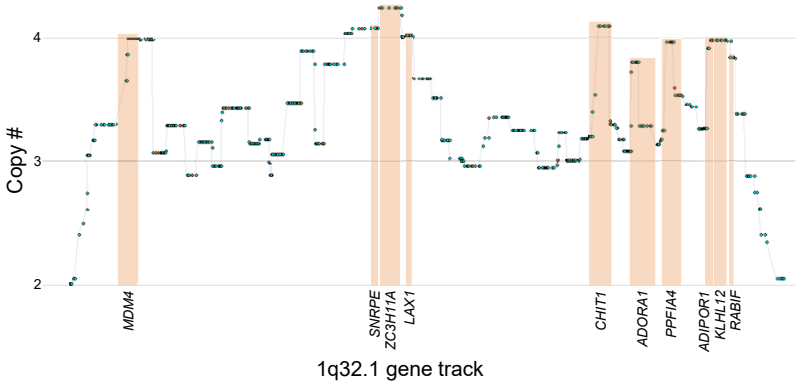

Figure 4. Microarray results of the amplified region on chromosome 1q32.1 exhibit an emerging stepwise pattern. Gene regions are selectively included based on functionality and overlap of copy number peaks.

References

1) Baker, D. et al. Detecting genetic mosaicism in cultures of human pluripotent stem cells. Stem Cell Rep. 7, 998–1012 (2016).
2) Albertson, D.G. et al. Quantitative mapping of amplicon structure by array CGH identifies CYP24 as a candidate oncogene. Nature Genet. 25, 144–146 (2000).
3) Avery, S. et al. BCL-XL mediates the strong selective advantage of a 20q11.21 amplification commonly found in human embryonic stem cell cultures. Stem Cell Rep. 1, 379–386 (2013).
4) O'Leary NA, et al. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res. Jan 4;44(D1):D733-45 (2016).