SUPPLEMENTAL INFORMATION

*September 2, 2005*

# Genetic and Epigenetic Modeling of the Origins of Multidrug Resistant Cells in a Human Sarcoma Cell Line

Kevin G. Chen[1, 2, ✉], Yan C. Wang[1], Marci E. Schaner[1, 2], Brian Francisco[1], George E. Durán[1], Dejan Juric[1], Lyn M. Huff[3], Hesed Padilla-Nash[4], Thomas Ried[4], Tito Fojo[3], and Branimir I. Sikic[1, 2]

[1]Division of Oncology, Department of Medicine, and [2]the Program in Cancer Biology, Stanford University School of Medicine, Stanford, CA 94305-5151, USA; [3]Medicine Branch, and [4]Genetics Branch of the National Cancer Institute, National Institutes of Health, Bethesda, MD, 20892, USA

**Running Title**: Origins of Multidrug Resistant Cells

**Key Words:** Genomic instability, spontaneous mutations, multidrug resistance, *ABCB1*, histone acetylation

**Footnotes**: This investigation was supported by the National Institutes of Health grant R01-CA92474 (to B.I.S.) and National Cancer Institute grant CA09302 (to both K.G.C. and M.E.S.).

✉Correspondence or requests for reprints: Current address: Kevin G. Chen, M.D., Ph.D, Stem Cell Unit, National Institute of Neurological Disorders and Stroke, NIH, Room 1000, Building 37, Convent Drive, Bethesda, MD, 20892; Phone: 301-402-8118; Fax: 301-480-1022; E-mail: cheng@mail.nih.gov

**Legends to Supplementary Figures**

**Supplementary Figure 1:** Flow cytometric analysis of induction of functional P-glycoprotein (P-gp) in MES-SA cells treated by either sublethal doxorubicin or etoposide. Rh-123 staining was done in the presence (**5-8**) or absence (**1-4**) of the P-gp inhibitor PSC-833 (PSC, 2 μM) and analyzed by the FlowJo software. MES-SA/DOX6D and MES-SA/VP6D cells were derived by exposure of MES-SA cells to 40 nM doxorubicin and 0.5 μM etoposide for one week, respectively. MES-SA and Dx5 cells were used as negative and positive controls for P-gp function, respectively**.**

**Supplementary Figure 2:** Confirmation of far upstream sequences by 5'-RACE in the DSM: Top panel, The 202-bp 5'-RACE fragment (RACE202) from the DSM (10B-E2) was isolated by 5'-RACE protocols, which is composed of 70 bp of exon 1a, 94 bp of exon -1, and an additional 38 bp upstream of exon -1. Lower panel, Sequences of the 5-RACE product from 10B-E2 cells (Accession number: AF345625) were homologous to two regions of the BAC clone CTB-060P12 from 7q21: 28422-28553 and 140636-140705. The exon -1 sequences were gray color-highlighted. Abbreviations: AUAP, abridged universal anchor primer; GSP, gene specific primer.

**Supplementary Figure 3: (**Upper panel) RT-PCR analysis of induction of both ut370 and tm325 mRNAs by short-term exposure of MES-SA cells to 80 nM doxorubicin at the indicated times. (Lower panel) RT-PCR analysis of induction of tm325 in MES-SA cells by escalated doxorubicin concentrations as indicated. Dx5 cells were used positive control for tm325, whereas MFS/VL20-4.1 was used as a positive control for both tm325

and ut370. rRNA was used as the control gene to normalize sample loading. The samples were analyzed by a 2% agarose gel and stained with ethidium bromide.

**Supplementary Notes**

**Supplementary Notes to Figure 2. *A*,** We extended Southern blotting analysis to at least four other DSMs with different probes and obtained the same results as shown in Figure 2 (data not shown). Moreover, the *ABCB1* promoter regions (-1018 to +83) in the DSMs (2B-E3, 3B-B9, and 10B-E2) have been amplified by PCR, sublconed, and sequenced. No mutations or gene rearrangements have been found in this area (data not shown). Thus, *cis*-genomic alterations (i.e., gene amplification, gene rearrangement, or mutations) within the *ABCB1* promoter region are not associated with *ABCB1* activation in these DSMs. ***E***, In the calculation for doxorubicin selection from Figure 2E, two clones each were analyzed from populations 2 (2B-E3, 2B-F5), but these are scored as representing sister clones of one positive mutant with upstream sequences from each population. Transcripts with the upstream sequences (ut370) originating at the upstream site were not found in MES-SA parental cells (after 35 cycles of PCR amplification), two multistep selected MDR lines MES-SA/Dx5 (Figure 2E, lane 3), and MCF-7/ADR (data not shown) which express high levels of *ABCB1*.