The Effect of Hoechst 33342 and Hoechst 33258 on Side Population (SP) Cells or Stem Cell-Like Population

Naimei Tang [1,2,5], Crystal Zhang[1], Cynthia Noraian[2], Anil Wali[3], Harvey Pass[4], Michael Harbut[2], and Xinbo Zhang[1]

[1] Internal Medicine, Wayne State University, Detroit, MI, [2] National Center for Vermiculite and Asbestos-Related Cancers (NCVAC), Karmanos Cancer Institute, Detroit, MI, [3]Center to Reduce Cancer Health Disparities, National Cancer Institute, Bethesda, MD. [4]NYU Langone Medical Center, New York, NY, [5] Microelectronic Engineering Department, University of Texas, Austin, TX

ABSTRACT

Background: Hoechst 33342 side **population** (SP) analysis is widely used for identifying and sorting side population (SP) or stem cell-like population from a variety of tissues and species including cancer cell lines. However, Hoechst 33342, but not its derivative Hoechst 33258, is an apoptotic inducer. Our previous results showed that Hoechst 33342 induced apoptosis in seven mesothelioma cell lines through cytochrome C release, caspase activation and degradation of Poly (ADP-ribose) polymerase.

Hypothesis: Hoechst 33342 may cause SP cell death during Hoechst 33342 staining and Hoechst 33258 may be a safe indicator for identification and isolation of SP cells.

The aims of this study are 1) to detect the effect of H 33342 on SP cells, and 2) to identify if H33258 can be an indicator for stem cell isolation.

Procedure: The effect of Hoechst dyes on the tumourigenicity of H2373 mesothelioma cells was analyzed by soft agar clonogenic colony formation. MTT assay have been employed to estimate the effect of Hoechst dyes on cell growth in H2373 mesothelioma cells, and Hoechst 33342 and Hoechst 33258 SP analysis was performed on a FACSDiVa cell sorter.

Our results demonstrated that Hoechst 33342 staining can causes SP cell damage and also decreases cell colony formation and cell proliferation after using the routine staining doses and intervals for SP cell analysis. In contrast, Hoechst 33258 has little effect on the cell colony formation and cell proliferation. We have found that verapamil, ABC transporter inhibitor, significantly inhibits the efflux of intracellular Hoechst 33258, which means Hoechst 33258 may be used as a marker in isolating ABC transporter positive cells, as Hoechst 33342 does.

CONCLUSION

1) Hoechst 33342 can induce apoptosis of SP cells of mesothelioma cancer cells
2) Hoechst 33258 may be a safe indicator and replace Hoechst 33342 for identification and isolation of side population.

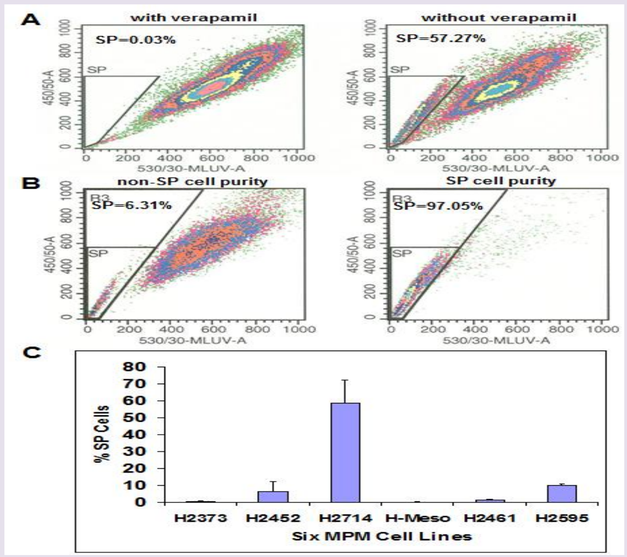

Figure 1. Hoechst 33342 SP cell analysis and prevalence of SP cells in six mesothelioma cell lines.
A, H2714 cells were stained with 5 µg/ml Hoechst 33342 dye for 90 min in the presence (left) or absence (right) of 50 µmol/L verapamil and analyzed by flow cytometry. B, the purities of sorted non-SP cells (left) and SP cells (right) were determined by flow cytometry. C, the prevalence of SP cells in six mesothelioma cell lines was determined by Hoechst 33342 SP cell analysis.

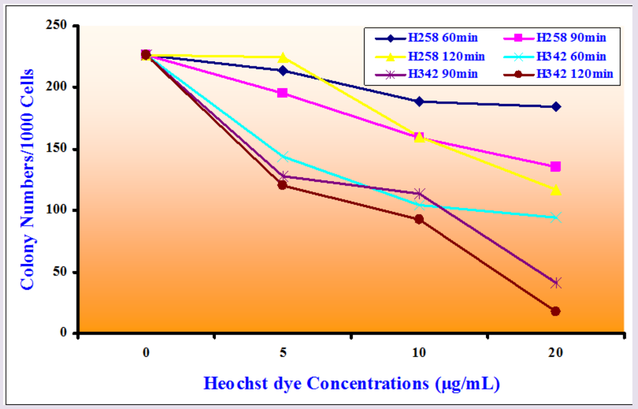

Figure 2. The effect of Hoechst dyes on the cell colony formation in H2373 cells.
H2373 cells were treated with H342 or H258 at different doses (0, 2.5, 5, 10 µg/ml) for various treatment times (60, 90, 120 min). Hoechst dyes were removed after the treatment and 1000 cells were mixed with medium containing 0.4% agarose and continued to culture for 3 weeks.

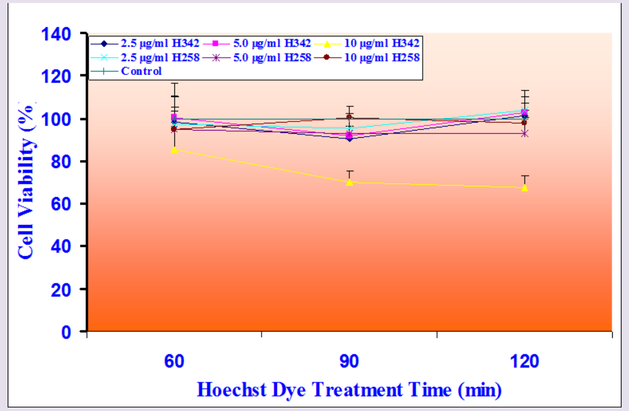

Figure 3. The effect of Hoechst dyes on the proliferation of H2373 cells.
H2373 cells were treated with H342 or H258 at different doses (0, 2.5, 5, 10 µg/ml) for various treatment times (60, 90, 120 min). Hoechst dyes were removed after the treatment and the cells were continued to culture for 1 day. Cell proliferation was measured by MTT assay. Each value indicates mean + SD.

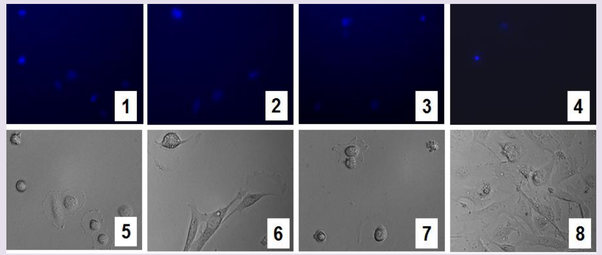

Figure 4. Hoechst 33342 exclusion capacity is associated with proliferation of SP and non-SP cells of MPM H2314cells after sorting for different times of culture.
1, 2, 3, 4, 5, 6, 7, and 8 indicate the changes in intensity of fluorescence and proliferation of non-SP cells (1, 2, 5 and 6) and SP cells (3, 4, 7 and 8) from 0 (1, 3, 5 and 7) to 2 days (2, 4, 6 and 8). 1 and 5, 2 and 6, 3 and 7, 4 and 8 are paired images.

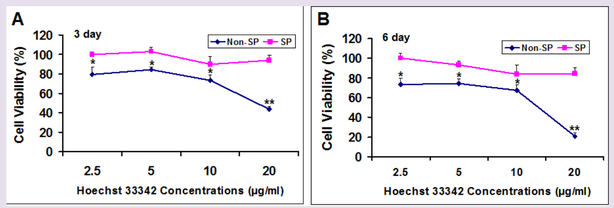

Figure 5. Hoechst 33342 decreases the proliferation and growth of non-SP cells

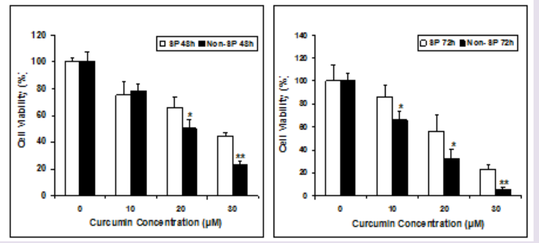

Figure 6. Hoechst 33342 SP cell analysis and prevalence of SP cells in six mesothelioma cell lines.
A, H2714 cells were stained with 5 µg/ml Hoechst 33342 dye for 90 min in the presence (left) or absence (right) of 50 µmol/L verapamil and analyzed by flow cytometry. B, the purities of sorted non-SP cells (left) and SP cells (right) were determined by flow cytometry. C, the prevalence of SP cells in six mesothelioma cell lines was determined by Hoechst 33342 SP cell analysis.

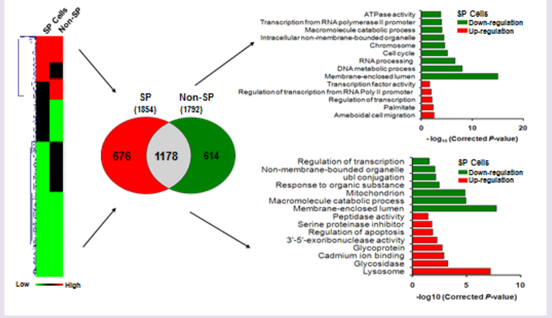

Figure 7. Hoechst 33342 SP cell analysis and prevalence of SP cells in six mesothelioma cell lines.
A, H2714 cells were stained with 5 µg/ml Hoechst 33342 dye for 90 min in the presence (left) or absence (right) of 50 µmol/L verapamil and analyzed by flow cytometry. B, the purities of sorted non-SP cells (left) and SP cells (right) were determined by flow cytometry. C, the prevalence of SP cells in six mesothelioma cell lines was determined by Hoechst 33342 SP cell analysis.

FUTURE STUDIES

1) More *in vitro* methods such as clonogenic assay, drug resistant assay, and invasion assay are required for further determination of stem cell-like properties of side population isolated by Hoechst 33258 when compared to that isolated by Hoechst 33342.
2) Xenograft (or animal) study is ultimately required to determine the capacity of *in vivo* tumor formation of the side population cells isolated by Hoechst 33258 after the completion of *in vitro* study