CURIOSIS

APPLICATION NOTE

Evaluating NK-92 cell-mediated cytotoxicity: A comparative analysis of suspension and adherent cancer cells using Celloger® Pro

■ Introduction

Solid tumor cells primarily exist in an adherent state, attaching to specific tissues and leading to cancers such as those of the breast, lung, and colon. However, some tumor cells can lose adhesion and undergo epithelial-to-mesenchymal transition (EMT), transforming into circulating tumor cells (CTCs). This transition enhances their motility and invasiveness, allowing them to enter the bloodstream and drive cancer metastasis.[1,2]

Natural killer (NK) cells play a crucial role in the innate immune system by identifying and eliminating abnormal cells. One of their key functions is targeting cells with reduced expression of Major Histocompatibility Complex (MHC) class I molecules, a hallmark of many tumor cells.[3,4] Notably, CTCs often exhibit low MHC class I levels and lack inhibitory ligands, making them prime targets for NK cell-mediated cytotoxicity.[4]

Although CTCs originate from the same primary tumor as solid tumor cells, they are detached from the extracellular matrix (ECM), leaving them directly exposed to the immune system. Consequently, CTCs exhibit distinct mechanical properties compared to adherent tumor cells, largely due to differences in their microenvironment.[5] These variations may influence their susceptibility to NK cell-mediated cytotoxicity. However, this relationship remains underexplored.

To address this, we investigated how the physical state - whether adherent or in suspension - of solid tumor cells affects NK cell-mediated cytotoxicity using Celloger® Pro, an automated live-cell imaging system. With its high-resolution, time-lapse imaging capabilities, Celloger® Pro enabled real-time visualization of tumor-immune interactions, providing a powerful tool for investigating cytotoxic mechanisms and advancing immunotherapeutic strategies.

■ Materials and Methods

Target cell preparation and staining

U-2OS cells were used as target cells under two experimental conditions: adherent and suspension states. For the adherent condition, U-2OS cells were seeded in a 48-well plate at a density of 3 × 10^4 cells per well and incubated at 37°C for 24 hours. The cells were then stained with 3 μM CellTracker™ Green CMFDA dye (Invitrogen, C2925) in serum-free medium for 30 minutes at 37°C, followed by washing with serum-free medium. For the suspension condition, detached U-2OS cells were stained under the same conditions and then seeded in the 48-well plate at the same density immediately after staining.

Co-culture and viability staining

After preparation, U-2OS cells were co-cultured with NK-92 cells (effector cells) at effector-to-target ratios of 5:1, 10:1, and 20:1. To assess cell viability, 4 μM Ethidium Homodimer-1 (EthD-1) (Sigma, 46043-1MG-F) was added to the co-cultured cells and incubated at room temperature for 15 minutes before imaging.

Live-cell imaging and analysis

Time-lapse imaging was performed using Celloger® Pro, capturing images every 1 hour for 24 hours with a 4X objective lens. Image analysis was performed using Celloger® Pro software to quantify green fluorescence from live cells and red fluorescence from dead cells, thereby evaluating NK cell-mediated cytotoxicity.

■ Result

NK cell-mediated cytotoxicity was analyzed using NK-92 cells as effector cells and U-2OS cells as target cells. To examine how the physical state of target cells influences NK-92 cell-mediated cytotoxicity, U-2OS cells were prepared under either adherent or suspension conditions. In the adherent condition, U-2OS cells were seeded and cultured for 24 hours before staining with CellTracker™ CMFDA dye, which emits green fluorescence. For the suspension condition, cells were detached, stained, and immediately reseeded. NK-92 cells were then added to both conditions along with EthD-1, a viability dye that fluoresces red when binding to DNA in dead cells. This enabled the assessment of NK cell cytotoxicity and target cell viability.

Time-lapse imaging at 1-hour intervals over 24 hours using Celloger® Pro enabled continuous monitoring of NK-92 cell-mediated cytotoxic effects and cell-cell interaction dynamics. Image analysis with Celloger® Pro revealed distinct cytotoxicity patterns between adherent and suspension conditions, with time-dependent differences in NK-92 cell activity (Figure 1).

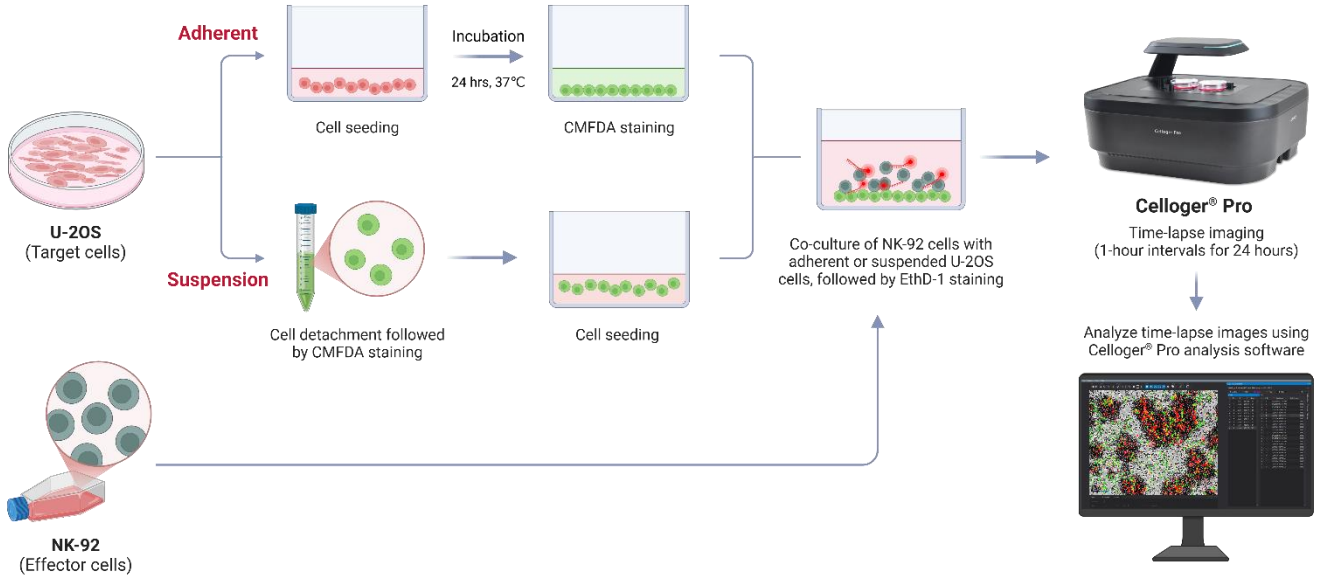

Figure 1. Experimental workflow for NK cell-mediated cytotoxicity assay using Celloger® Pro

In the adherent condition, green fluorescence from U-2OS cells gradually decreased as the effector-to-target (E:T) ratio increased. At higher ratios, particularly 20:1, NK-92 cells extensively accumulated around the target cells, resulting in increased red fluorescence and enhanced cytotoxicity (Figure 2A). A similar pattern was observed under suspension conditions. NK-92 cells rapidly clustered around target cells at a 20:1 ratio, causing a sharp decline in green fluorescence and a corresponding rise in red fluorescence, indicative of heightened cytotoxic activity (Figure 2B). To quantitatively assess NK cell-mediated cytotoxicity, the red-to-green fluorescence intensity ratio was measured over the 24-hour period. The analysis revealed a time- and ratio-dependent increase in cytotoxic effects in both adherent and suspension conditions (Figure 2C, 2D).

To compare the responses of adherent and suspension U-2OS cells to NK-92 cell-mediated cytotoxicity, time-lapse images were cropped at an E:T ratio of 10:1 and analyzed at 0, 12, and 24 hours to observe the distribution of green and red fluorescence (Figure 3A). In the adherent condition, green fluorescence from U-2OS target cells gradually decreased over time, indicating a progressive reduction in cell viability. In contrast, cytotoxicity in the suspension condition was more rapid and pronounced. A sharp decline in green fluorescence was observed by 12 hours, accompanied by a substantial increase in red fluorescence, reflecting an accelerated cytotoxic response mediated by NK-92 cells. This trend was quantitatively confirmed in Figure 3B, where the red-to-green fluorescence intensity ratio increased more rapidly and reached higher levels in suspension cells compared to adherent cells at the same E:T ratio of 10:1. These results suggest that U-2OS target cells in suspension are more susceptible to NK-92 cell-mediated cytotoxicity, as demonstrated by the faster loss of green fluorescence and the greater increase in red fluorescence.

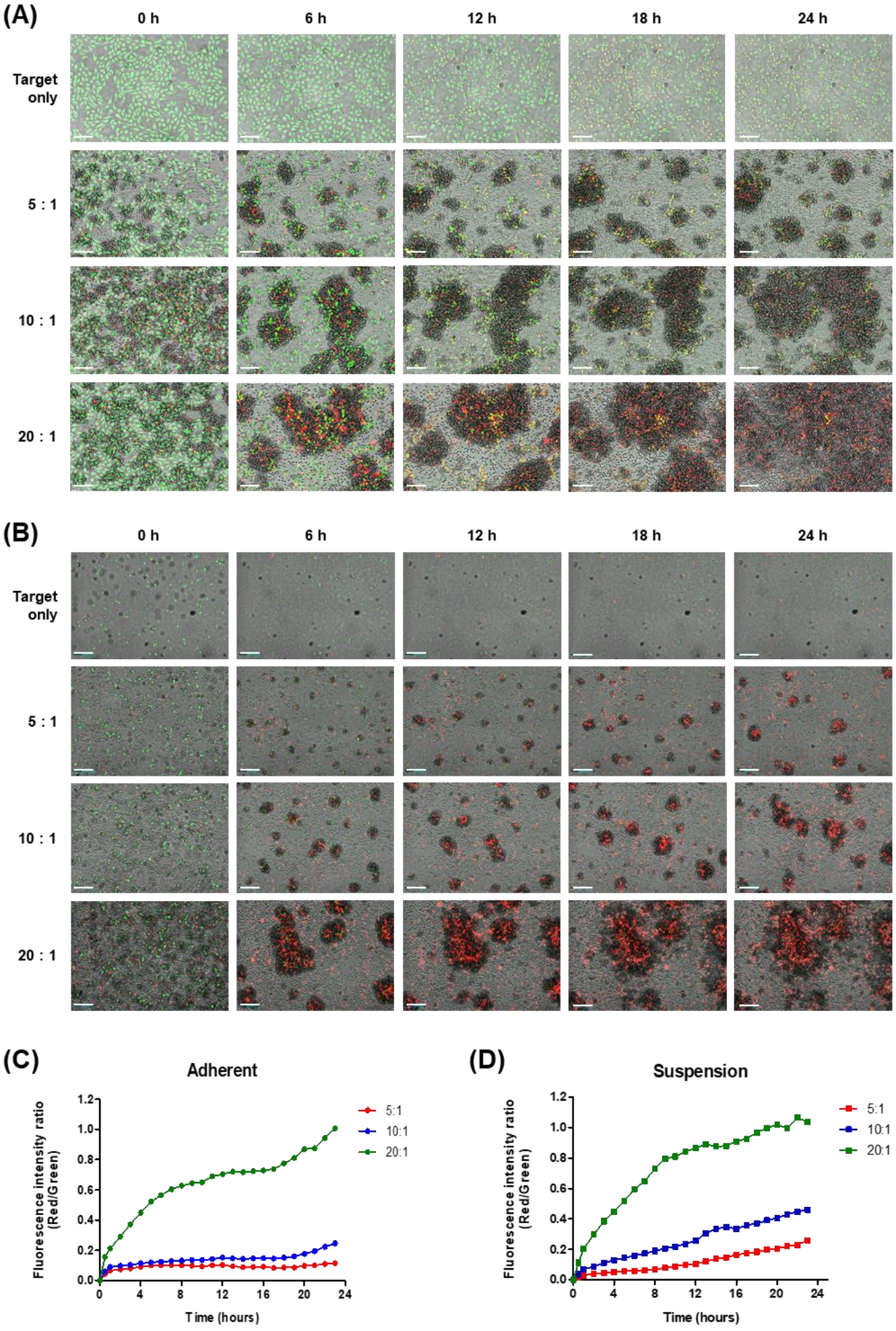
(A)
0 h
6 h
12 h
18 h
24 h
Target only
5 : 1
10 : 1
20 : 1
(B)
0 h
6 h
12 h
18 h
24 h
Target only
5 : 1
10 : 1
20 : 1
(C)
Adherent
Fluorescence intensity ratio (Red/Green)
Time (hours)
5:1
10:1
20:1
(D)
Suspension
Fluorescence intensity ratio (Red/Green)
Time (hours)
5:1
10:1
20:1

Figure 2. Time-lapse imaging of NK cell-mediated cytotoxicity in adherent and suspension target cells using Celloger® Pro

U-2OS target cells were labeled with CMFDA green dye to visualize viable cells, while EthD-1 red dye was used to stain dead cells. **(A, B)** NK-92 effector cells were co-cultured with adherent (A) and suspended (B) U-2OS cells. Real-time imaging was performed over 24 hours at different E:T ratios (5:1, 10:1, and 20:1), with the "target only" group serving as the control. Scale bar = 200 µm. **(C, D)** Graphs show the red-to-green fluorescence intensity ratio of adherent (C) and suspended (D) U-2OS cells. Quantitative analysis was performed using Celloger® Pro analysis software.

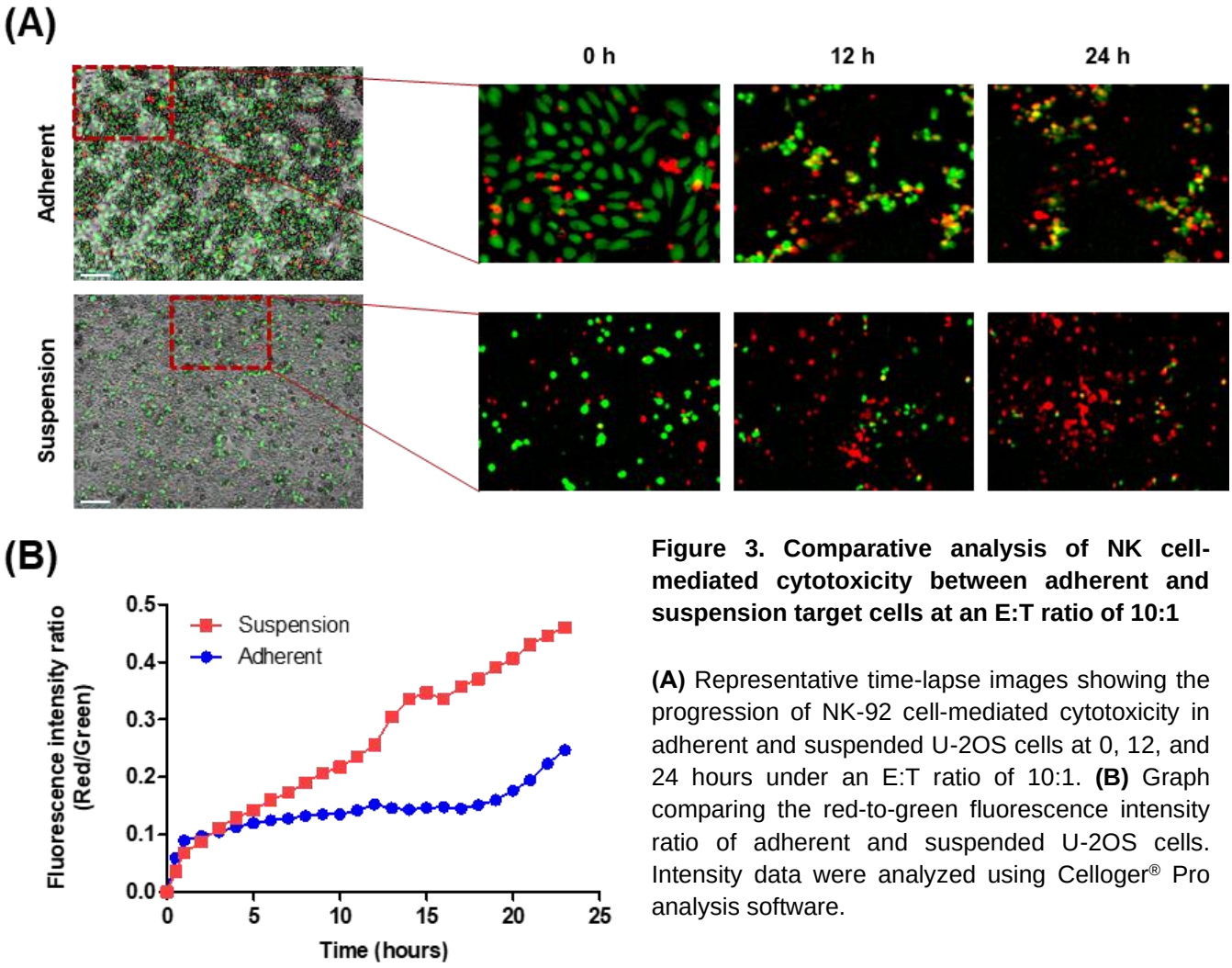

Figure 3. Comparative analysis of NK cell-mediated cytotoxicity between adherent and suspension target cells at an E:T ratio of 10:1

(A) Representative time-lapse images showing the progression of NK-92 cell-mediated cytotoxicity in adherent and suspended U-2OS cells at 0, 12, and 24 hours under an E:T ratio of 10:1. **(B)** Graph comparing the red-to-green fluorescence intensity ratio of adherent and suspended U-2OS cells. Intensity data were analyzed using Celloger® Pro analysis software.

■ Conclusion

In this application note, we demonstrate that U-2OS cells in suspension are more susceptible to NK cell-mediated cytotoxicity than those in adherent conditions. Several factors may contribute to this difference. Cell adhesion plays a key role in regulating NK cell-activating ligand expression, influencing NK cell recognition and targeting.[6] Additionally, the higher density of exposed functional surface molecules on suspension cells may promote interactions with NK cells, increasing their susceptibility to immune attack. Metabolic differences between adherent and suspension cells could also play a role in their varying sensitivity,[7,8] though further studies are needed to confirm this in osteosarcoma.

By utilizing Celloger® Pro, we efficiently visualized and quantified cell viability in real time using a two-color fluorescence-based approach. This streamlined and reliable cytotoxicity assessment method highlights the potential of Celloger® Pro as a powerful tool for researchers studying NK cell-based immunotherapies. Its ability to provide high-resolution, real-time imaging of immune cell interactions and target cell viability makes it an essential asset for advancing research in immune-oncology.

■ References

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