Toxicity mediated by some 293T packaging cell lines

During the course of our studies, we noticed that the health of the 293T packaging cell line was crucial to produce good viral titers. We obtained lower titers when we used cells that had been grown for several passages (>20) or that were too confluent at the time of the transfection, or when we used old OPTI-MEM that had been warmed up too many times. All these variables have already been described previously [1]. However, we noticed another effect with a particular batch of 293T cells that, to our knowledge, was not previously characterized. Viruses derived from these 293T cells induced senescence in WI38 primary fibroblasts and changed the morphology of U2OS osteosarcoma cells from their normal orthogonal shape (Figure 1A) into an elongated "spindle" shape (Figures 1B, C).

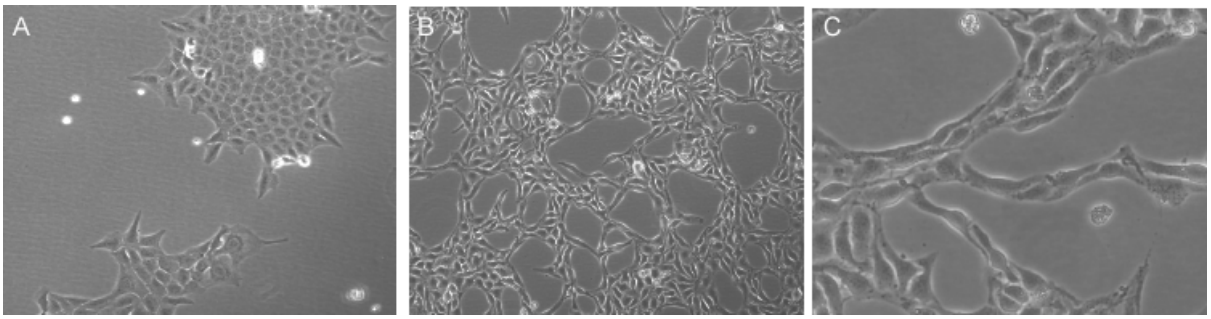


Figure 1. Some isolates of 293T packaging cells can affect the morphology of U2OS osteosarcoma cells. (A) untransduced U2OS cells. (B) U2OS cells incubated 72 hours with conditioned media from 293T cells (C) Higher magnification of panel (B).

This change in morphology was gradual, often arising as an initial colony in the population that was gradually spreading to all the cells (Figure 2). Furthermore, once some U2OS cells in a population displayed this change of morphology, they could induce neighboring, untransduced U2OS cells to change their morphology, implying a secreted factor. To test this hypothesis, we took media from orthogonal or spindle-shaped non-GFP-expressing U2OS cells, filtered the media through a 0.2 μm syringe filter to remove residual cells, and incubated it with an U2OS-GFP cell line. As shown in Figures 3A and 3B, media derived from orthogonal U2OS cells did not alter the morphology of the U2OS-GFP cells whereas media derived from the spindle-shaped cells induced a spindle shape morphology in U2OS-GFP cells. We also found that culture media from the initial "unhealthy" 293T cell line could promote this change in U2OS cells. This "serial passaging" of the toxic effect supported our idea the factor initially secreted by the 293T packaging cell line could also promote U2OS cells to secrete the same or a similar factor. Concentration of the virus by a dual ultracentrifugation did not prevent the phenotype (result not shown), even though most of the media was removed during this step. This toxic effect could be seen with lentiviruses regardless if they were from the second or third generation as well as with retroviruses, arguing again that it was the 293T packaging cell line and not the virus that was responsible for the toxic effect. We obtained 293T cells from another source (a kind gift from Christian Beauséjour) and these cells did not show the toxic

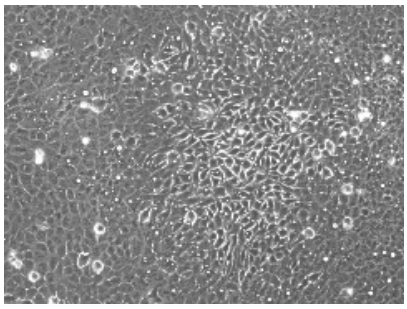


Figure 2. Altered morphology of a subset of cells in a population 2 days after incubation with the conditioned media.

phenotype. Other cell lines such as HeLa, HCT116 or A549 cells did not display this change in morphology when they were treated under the same conditions that induced a morphological change in U2OS cells, although we could not rule out a more subtle effect we did not detect (results not shown). This demonstrates the importance of monitoring passage number and health of the 293T packaging cell line as well as the use of either an empty viral vector or a vector encoding an irrelevant cDNA/shRNA/miRNA as negative controls. We do not know if the difference between the lots of 293T cells is due to passage number or clonal drift, but the change in U2OS morphology is an easy assay to monitor for the presence of such a stress factor that might affect the outcome of experiments.

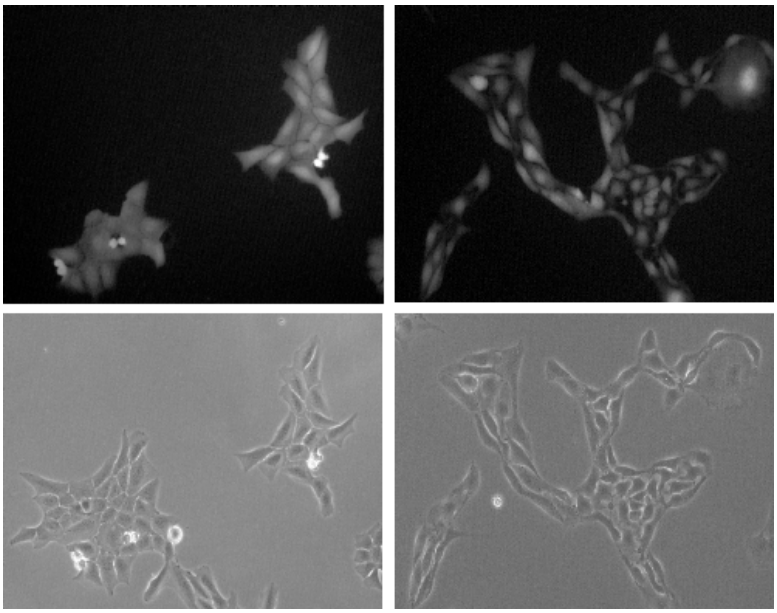


Figure 3. The toxic effect is secreted and can be propagated in U2OS cells. LEFT: GFP-U2OS cells incubated with media conditioned from untransduced, orthogonal-shaped, non-GFP U2OS cells. RIGHT: GFP-U2OS cells incubated with media conditioned from untransduced, spindle-shaped non-GFP U2OS cells. TOP PANELS represent GFP channel whereas the BOTTOM PANELS is the bright field version of the same picture.

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1. **Frederick M. Ausubel RB, Robert E. Kingston, David D. Moore, J.G. Seidman, John A. Smith,, Struhl K**, editors (2003) Current Protocols in Molecular Biology: **John Wiley & Sons, Inc.** 4648 p.