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LETTER TO THE EDITOR

Herpes simplex virus type 1 suppresses the transformed phenotype of cultured cells

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It has been shown decades ago, that herpes simplex viruses type 1 and 2 (HSV-1,-2) after irradiation with UV light or after photoinactivation in the presence of a photosensitizer, e.g. neutral red, are capable of transforming cells *in vitro* (*1,2,3,4*), and similar activity was later demonstrated with UV-irradiated and photoinactivated murine gammaherpesvirus 68 (MHV-68)(*5,6*).

Recently, Michútová *et al.* (*7*) continued the studies of these phenomena and obtained interesting original results. Their work suggests that only non-syncytial strains of HSV-1 are capable of transforming cells *in vitro* after photoinactivation in the presence of methylene blue, such transforming activity has not been demonstrated for photoinactivated syncytial strains.

Another interesting phenomenon was observed in transformed cell lines. Photoinactivated non-syncytial but not syncytial strains of HSV-1 have been shown to have the ability to suppress the transformed phenotype of cells so that their morphology resembled that of non-transformed cells. In an attempt to demonstrate the more general validity of this new phenomenon, we decided to repeat these experiments with UV-irradiated strains of HSV-1.

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Abbreviations: HSV-1,2 = herpes simplex virus type 1, 2; MHV-68 = mouse herpesvirus 68

Two non-syncytial HSV-1 strains, KOS and Kupka, and two syncytial strains, HSZP and ANGpath, were selected for our studies. The detailed procedure for irradiating viruses with UV light has been described in our previous paper and the inactivation process was similar to that of MHV-68 (*5*). Inactivated virus samples were tested in non-transformed human diploid lung embryonic (MRC-5) cells and mouse fibroblast (NIH3T3) cells as well as in transformed mouse liver hepatoma (Hepa) cells and in human immortalized keratinocytes HaCaT. The cells were cultivated in Dulbecco´s modified Eagle Medium supplemented with 7% fetal bovine serum, 1% L-glutamine and 1% Penicillin/Streptomycin/Amphotericin. The titres of all virus samples were adjusted to $1x10^6$ PFU/ml, then irradiated by UV light for 30 s and added to cells at a multiplicity of infection of 0.01 of non-irradiated virus (*5*).

The process of transformation in non-transformed MRC-5 and NIH3T3 cells followed similar pattern as described previously (*1,5,6*). The transformation of non-transformed cells by inactivated HSV is already well known for years and relatively well understood, so we have not studied it in details now. But we confirmed the results recently obtained with photoinactivated HSV-1 strains. Only UV-irradiated non-syncytial strains KOS and Kupka proved to be able to transform the cells. The cells infected with irradiated syncytial strains HSZP and ANG path remained intact without any morphological changes similar to those infected with photoinactivated syncytial strains (*7*). The cells infected with both UV-irradiated syncytial and non-syncytial strains

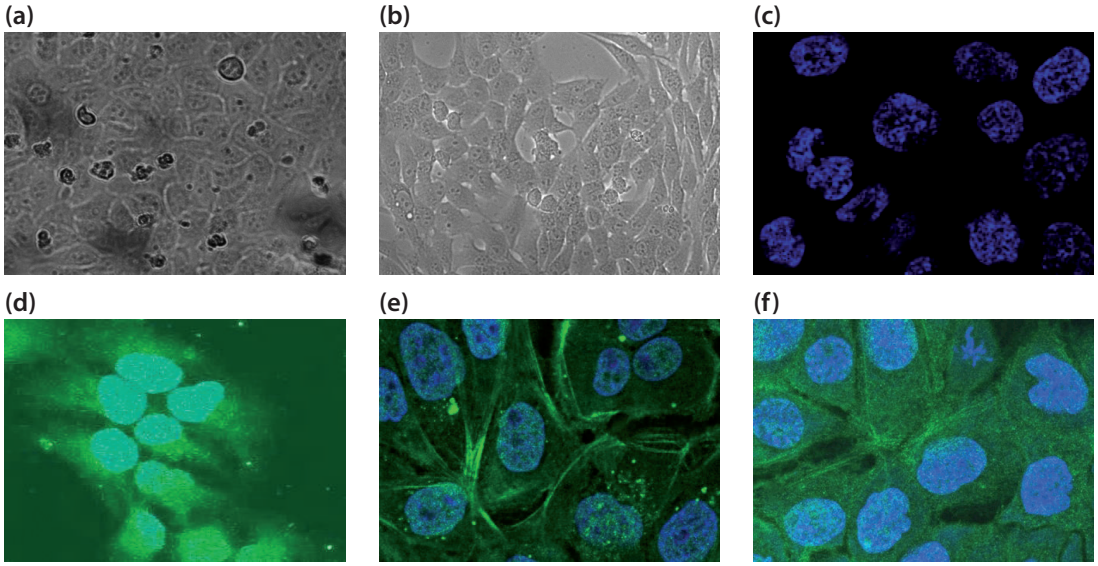

Fig. 1

Infection of HaCaT cells with UV-irradiated virus

(a) control cells; **(b)** cells in 3rd passage following infection with UV-irradiated HSV-1, strain KOS; **(c)** control cells stained with Dapi; (**d**) fluorescently stained virus antigen in cells infected with UV-irradiated Kupka strain of HSV-1 (2. passage); **(e)** control cells showing actin bundles; (**f**) cells after infection with UV-irradiated KOS strain of HSV-1 showing disappearance of actin bundles.

contained virus antigen as demonstrated after staining with FITC anti-HSV1,2 goat polyclonal antibody (Invitrogen) (data not shown).

The effect of UV-irradiated non-syncytial and syncytial HSV-1 strains on transformed cells was studied in more details. These cells, about 24 h after infection with UV-irradiated non-syncytial strains lost their transformed phenotype, which resembled the non-transformed one.

Fig. 1a shows HaCaT control cells, and in Fig. 1b are the same cells after infection with UV-irradiated HSV-1 strain KOS. The altered phenotype of transformed cells proved to be stable during cell passaging and virus cytopathic effect (CPE) did not appear in the cells after 10 and more passages, thus indicating that the cells did not contain infectious virus. However, virus antigen has been shown in these cells after 3–5 passages by immunofluorescence, although fluorescence was less intensive than in the case of productive lytic infection (Fig. 1c,d shows control HaCaT cells with nuclei stained with DAPI). When the cells were stained with Alexa Fluor 555 Phalloidin (Invitrogen) normally developed actin bundles were observed in control HaCaT cells (Fig. 1e) but after HSV-1, KOS strain infection associated with the suppression of the transformed phenotype, disappearance of actin bundles was observed (Fig. 1f). Similar changes in the structure of actin bundles were obtained in cells infected with UV-irradiated or photoinactivated MHV-68 (*5,6*). In HaCaT cells infected with UV-irradiated syncytial ANGpath strain, with unchanged phenotype only viral fluorescence antigen similar to that shown in Fig. 1d was visible, but no changes in the actin filament structure were observed (data not shown). This whole procedure was repeated in mouse transformed Hepa cells and the results were the same.

As mentioned above, the ability of HSV-1 and -2 to transform cells after UV irradiation or after photoinactivation has been known for decades (*1,2,3,4*) and has also been recently demonstrated with MHV-68 (*6,7*). However, what is new and has been demonstrated in our previous studies (*7*) and in now presented results is the fact that such inactivated viruses, whether UV-irradiated or photoinactivated in the presence of methylene blue (*7*) behave completely differently in transformed cells. They change their transformed phenotype towards the normal one, so that these cells resemble normal, non-transformed, contact inhibited cells (Fig. 1a,b). The alteration of the transformed phenotype that proved to be stable, did not change during passaging of cells and infectious virus (CPE) did not appear after more than 10 passages. Cells with altered transformed phenotypes showed changes in the actin filament structure, thus suggesting that these phenotypic changes may be stable. Another interesting finding is that the ability to transform cells or to alter the transformed phenotype of cells is restricted only to syn^- strains of HSV-1, i. e. the strains causing cell rounding, unable to form polycaryocytes (syncytia). This phenomenon was described in our previous study (*7*).

Virus-associated growth factors found in several herpes viruses are likely to be responsible for cell transformation

or alteration of the transformed phenotype (*7,8,9,10*). They are most likely been produced only by non-syncytial HSV-1 strains (*7*). It would be interesting to demonstrate a similar effect of inactivated non-syncytial HSV-1 strains on tumours *in vivo*. It could open a new way to the virotherapy of cancer.

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