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Brief Communication

Nuclear export of cutaneous HPV8 E7 oncoprotein is mediated by a leucine-rich nuclear export signal via a CRM1 pathway

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ABSTRACT

We recently determined that the nuclear import of cutaneous beta genus HPV8 E7 oncoprotein it is mediated by its zinc-binding domain via direct hydrophobic interactions with the FG nucleoporins Nup62 and Nup153 (Onder and Moroianu, 2014). Here we investigated the nuclear export of HPV8 E7 oncoprotein using confocal microscopy after transfections of HeLa cells with EGFP–8cE7 and mutant plasmids and treatment with Ratjadone A nuclear export inhibitor. We determined that HPV8 E7 contains a leucine-rich nuclear export signal (NES), $_{76}$**I**R**T**F**Q**E**LL**F$_{84}$, within its zinc-binding domain that mediates its nuclear export via a CRM1 pathway. We found that HPV8 E7 interacts with CRM1 and that the hydrophobic amino acid residues **I**76, **F**79 and **L**82 of the NES are essential for this interaction and for nuclear export of HPV8 E7 oncoprotein.

1. Introduction

Nonmelanoma skin cancer (NMSC) is the most common cancer in fair skinned populations. Exposure to UV radiation, along with fair skin, immune status and HPV infection, are the greatest risk factors (Akgul et al., 2006a; Feltkamp et al., 2008). A link between HPV infections and the development of skin cancer was first discovered in patients with the rare autosomal recessive disorder epidermodysplasia verruciformis (EV). Among the 14 types of cutaneous HPVs found in skin tumors of EV patients, the beta genus HPV 5 and 8 are specifically linked to malignant lesions and actinic keratoses and have been classified as high risk types (Akgul et al., 2006a; Bouwes Bavinck et al., 2008; Dubina and Goldenberg, 2009; Feltkamp et al., 2008; zur Hausen, 2009). Although EV patients are rare, the incidence of beta genus HPV associated squamous cell carcinoma (SCC) is highly increased in immunocompromised patients with beta-HPV DNA being detected in up to 90% of skin cancers of these individuals (Akgul et al., 2006a).

The HPV E7 proteins are acidic oncoproteins of approximately 98–103 amino acids that contain 3 domains: the conserved region (CR) 1, CR2, and the carboxyl terminal (C-terminal) domain. The CR1 domain is necessary for cellular transformation and RB degradation in mucosal high risk HPVs. The CR2 domain contains a conserved pRB family binding site (LxCxE domain) and a consensus casein kinase II (CKII) phosphorylation site (McLaughlin-Drubin and Munger, 2009). The C-terminal domain contains a zinc-binding domain that is composed of two Cys-X-X-Cys motifs, separated by 29–30 amino acids, and is involved in dimerization of E7 proteins and association with host complexes (Jones and Munger, 1996; McLaughlin-Drubin and Munger, 2009; Zwerschke and Jansen-Durr, 2000).

We previously discovered that the E7 oncoproteins of mucosal high risk HPV16 and low risk HPV11 enter the nucleus via a Ran-dependent pathway, independent of karyopherins/importins, and this pathway is mediated by their zinc-binding domain via hydrophobic interactions with the FG nucleoporins Nup62 and Nup153 (Angeline et al., 2003; Knapp et al., 2009; McKee et al., 2013; Eberhard et al., 2013; Piccioli et al., 2010). We also determined that HPV16 E7 and HPV11 E7 contain a leucine-rich NES within their zinc-binding domain that mediates their nuclear export via a CRM1 pathway (Knapp et al., 2009; McKee et al., 2013). CRM1 (chromosome region maintenance 1), a major nuclear export receptor, binds to hydrophobic leucine-rich NES containing cargoes in a RanGTP dependent manner. The canonical NES is defined as a L-X_{2-3}-L-X_{2-3}-L-X-L motif, where the leucine residues can be isoleucine, valine, phenylalanine or methionine (Hutten and Kehlenbach, 2007). The RanGTP/CRM1/NES cargo complex translocates through the nuclear pore complex and the cargo is released into the cytoplasm by GTP hydrolysis of RanGTP mediated by RanGAP (Hutten and Kehlenbach, 2007).

Although the activities, interactions and nucleocytoplasmic traffic of mucosal alpha genus HPV E7 oncoproteins have been well documented (McLaughlin-Drubin and Munger, 2009), much less is known about the cutaneous beta genus HPV E7 proteins. The cutaneous HPV8 E7 oncoprotein has a weaker interaction with pRB than HPV16 E7

Abbreviations: HPV, human papillomavirus; NLS, nuclear localization signal; NES, nuclear export signal; EGFP, enhanced green fluorescent protein; GST, Glutathione-S-transferase

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oncoprotein and can transform rodent cells only in collaboration with an activated H-ras gene (Yamashita et al., 1993). Expression of HPV8 E7 protein in primary human keratinocytes causes polyploidy that is associated with decreased levels of pRb and p21 (Akgul et al., 2007). It also causes invasion of human keratinocytes into the dermis of organotypic skin cultures that is accompanied by overexpression of extracellular metalloproteinases (Akgul et al., 2005, 2006b).

Flag-HPV8 E7 expressed in transfected human keratinocytes and EGFP-HPV8 E7 expressed in transfected HeLa cells are both predominantly localized in the nucleus with some low levels in the cytoplasm (Sperling et al., 2012; Onder and Moroianu, 2014). We recently determined that cutaneous beta genus HPV8 E7 oncoprotein contains a cNLS within its zinc-binding domain that mediates its nuclear import and localization independent of karyopherins/importins. Furthermore, we discovered that a mostly hydrophobic patch $_{65}$**LRLFV**$_{69}$ within the zinc-binding domain mediates nuclear import and localization of HPV8 E7 via direct hydrophobic interactions with the FG nucleoporins Nup62 and Nup153 (Onder and Moroianu, 2014).

In this study we investigated the nuclear export of cutaneous HPV8 E7 oncoprotein. We discovered and characterized a leucine-rich NES, $_{76}$**IRTFQELLF**$_{84}$, within the zinc-binding domain that mediates nuclear export of HPV8 E7 via a CRM1 pathway. We determined that HPV8 E7 interacts with CRM1 and that the hydrophobic amino acid residues **I**76, **F**79 and **L**82 of the NES are essential for this interaction and consequently for nuclear export of HPV8 E7 oncoprotein.

2. Results and discussion

We have previously characterized the leucine-rich NESs located within the zinc-binding domains of mucosal high risk HPV16 E7 ($_{76}$**IRTLEDLLM**$_{84}$) and low risk HPV11 E7 ($_{76}$**IRQLQDLLL**$_{84}$) mediating their nuclear export in a CRM1 dependent manner (Knapp et al., 2009; McKee et al., 2013). The zinc-binding domain of cutaneous HPV8 E7 oncoprotein also contains a leucine-rich sequence ($_{76}$**IRTFQELLF**$_{84}$) that has homology with these previously characterized NESs of mucosal HPV E7 proteins. To determine if HPV8 E7 oncoprotein can also undergo nuclear export we used transient transfections in HeLa cells with plasmids containing enhanced green fluorescent protein (EGFP) fused to 8cE7$_{43–103}$ and mutants, and Ratjadone A (RJA), a specific nuclear export inhibitor that blocks CRM1-mediated nuclear export of proteins containing leucine-rich NESs. RJA inhibition functions the same way as Leptomycin B (LMB): it covalently modifies a cysteine residue in the NES-binding pocket of CRM1, thereby inhibiting NES-binding (Hutten and Kehlenbach, 2007; Meissner et al., 2004). We analyzed the effect of RJA on the localization of two cysteine mutants, EGFP–8cE7 CC60AA and EGFP–8cE7 C91A, that we have previously shown to have a pancellular localization, in contrast with the nuclear localization of the wild type EGFP–8cE7 (Onder and Moroianu, 2014). As a positive control for RJA, we used EGFP-16E7–NES, which contains a fusion of 16E7 with the strong leucine-rich NES of HIV Rev at its C-terminus (Knapp et al., 2009). Wild type EGFP-16E7 localizes to the nucleus, and addition of the NES of Rev changes its localization to cytoplasmic (Knapp et al., 2009). HeLa cells were transfected with EGFP–8cE7, EGFP–8cE7 CC60AA, EGFP–8cE7 C91A or EGFP-16E7–NES and examined by confocal microscopy 24 h post transfection. The RJA treatment was performed after 20 h transfection for 4 h. Quantitative analysis of five independent experiments showed a change from $89.9 \pm 2.62\%$ pancellular localization for EGFP–8cE7 CC60AA mutant in the absence of RJA to $89.6 \pm 1.86\%$ nuclear localization in the presence of RJA (Fig. 1A, panels E and G; Fig. 1B). Also, the pancellular localization for EGFP–8cE7 C91A mutant in $88.4 \pm 1.98\%$ cells in the absence of RJA changed to $88.9 \pm 2.32\%$ nuclear localization in the presence of RJA (Fig. 1A, panels I and K; Fig. 1B). In the presence of RJA inhibitor the nuclear export of CC60AA and C91A mutants is inhibited which allows the accumulation of these mutants (with weakened NLS) in the nucleus at the steady state analyzed in the transfection assays. The nuclear localization of EGFP–8cE7 wild type remained largely unchanged after RJA treatment, with only a small increase in the percentage of cells with nuclear localization (Fig. 1A, panels A and C; Fig. 1B). As expected, the cytoplasmic localization of the EGFP-16E7–NES control changed to nuclear in the majority of transfected cells after RJA treatment (Fig. 1B). These data suggest the presence of a functional NES that is masked by a stronger NLS in the C-terminal domain of cutaneous HPV8 E7 oncoprotein.Similar to HPV16 E7 and HPV11 E7, HPV8 E7 protein has a leucine-rich NES sequence, $_{76}$**IRTFQELLF**$_{84}$, where the bold characters represent the hydrophobic amino acids that may be critical for the NES function. To characterize this NES sequence and the amino acids that are essential for its proper function, three NES mutants were generated in the context of EGFP–8cE7 C91A cysteine mutant: I76A, F79A and L82A. The C91A mutant was chosen for generating the three nuclear export mutants because it has only a minimal mutation that weakens the NLS of HPV8 cE7 and the RJA export inhibitor rescued its nuclear localization (Figs. 1A and 1B). HeLa cells were transfected with EGFP–8cE7, EGFP–8cE7 C91A, EGFP–8cE7 C91A/I76A, EGFP–8cE7 C91A/F79A, or EGFP–8cE7 C91A/L82A plasmids and the intracellular localizations of the expressed EGFP fusion proteins were analyzed 24 h after transient transfections using confocal fluorescence microscopy (Fig. 2). As expected, the majority of cells expressing EGFP–8cE7 showed mostly nuclear localization (Fig. 2, panels A and C, and Fig. 3), whereas EGFP–8cE7 C91A mutant exhibited pancellular localization (Fig. 2, panels D and F, and Fig. 3). Interestingly, the localization of EGFP–8cE7 C91A/I76A, EGFP–8cE7 C91A/F79A, and EGFP–8cE7 C91A/L82A NES mutants was changed to mostly nuclear (Fig. 2, panels G and I; J and L; M and O). Quantitative analysis of four independent experiments showed that all these three NES mutants exhibited predominant nuclear localization in the majority of cells similar as the wild type EGFP–8cE7 expressing-cells (Fig. 3). These data suggest that cutaneous HPV8 E7 has a functional leucine-rich NES within its C-terminal domain and that the hydrophobic amino acid residues, **I**76, **F**79 and **L**82 are essential for its nuclear export function.

The identification of a functional leucine-rich NES mediating 8E7 nuclear export led us to analyze the interaction between 8E7 and CRM1 nuclear export receptor. HeLa cell lysates containing EGFP-8E7, EGFP–8cE7, EGFP-16E7–NES (as a positive control), or EGFP (as a negative control) were incubated with GST-CRM1 or GST immobilized on glutathione-Sepharose beads. Cell lysates and the eluted bound proteins were analyzed via immunoblotting with an anti-GFP antibody. Both EGFP-8E7 and EGFP–8cE7 bound to GST-CRM1 and not to GST itself (Fig. 4A, lanes 1, 2, 5, 6, 9 and 10), as well as the positive control EGFP-16E7–NES (Fig. 4A, lanes 3, 7 and 11). EGFP did not interact with either GST-CRM1 or GST (Fig. 4A, lanes 4, 8 and 12). These data indicate that HPV8 E7 oncoprotein interacts with CRM1 nuclear export receptor via its zinc-binding domain.

To investigate the role of the leucine-rich NES in the interaction between 8cE7 and CRM1 we performed isolation assays with all three NES mutants in comparison with EGFP–8cE7 and EGFP–8cE7 C91A. We found that both EGFP–8cE7 and EGFP–8cE7 C91A bound to GST-CRM1 but not to GST (Fig. 4B, lanes 1, 2, 7, 8, 13 and 14). Interestingly, all three NES mutants, EGFP–8cE7 C91A/I76A, EGFP–8cE7 C91A/F79A and EGFP–8cE7 C91A/L82A did not interact with GST-CRM1 (Fig. 4B, lanes 3, 4, 5, 9, 10 and 11). As expected, EGFP did not interact with either GST-CRM1 or GST (Fig. 4B, lanes 6, 12 and 18). These results indicate that these NES mutations inhibit the interaction of HPV8 E7 with CRM1 supporting the critical role of the hydrophobic amino acids, **I**76, **F**79 and **L**82 for the nuclear export function.

Overall, our results indicate that the cutaneous HPV8 E7 oncoprotein has a leucine rich NES ($_{76}$**IRTFQELLF**$_{84}$) within the zinc-binding domain that interacts with CRM1 export receptor and mediates its

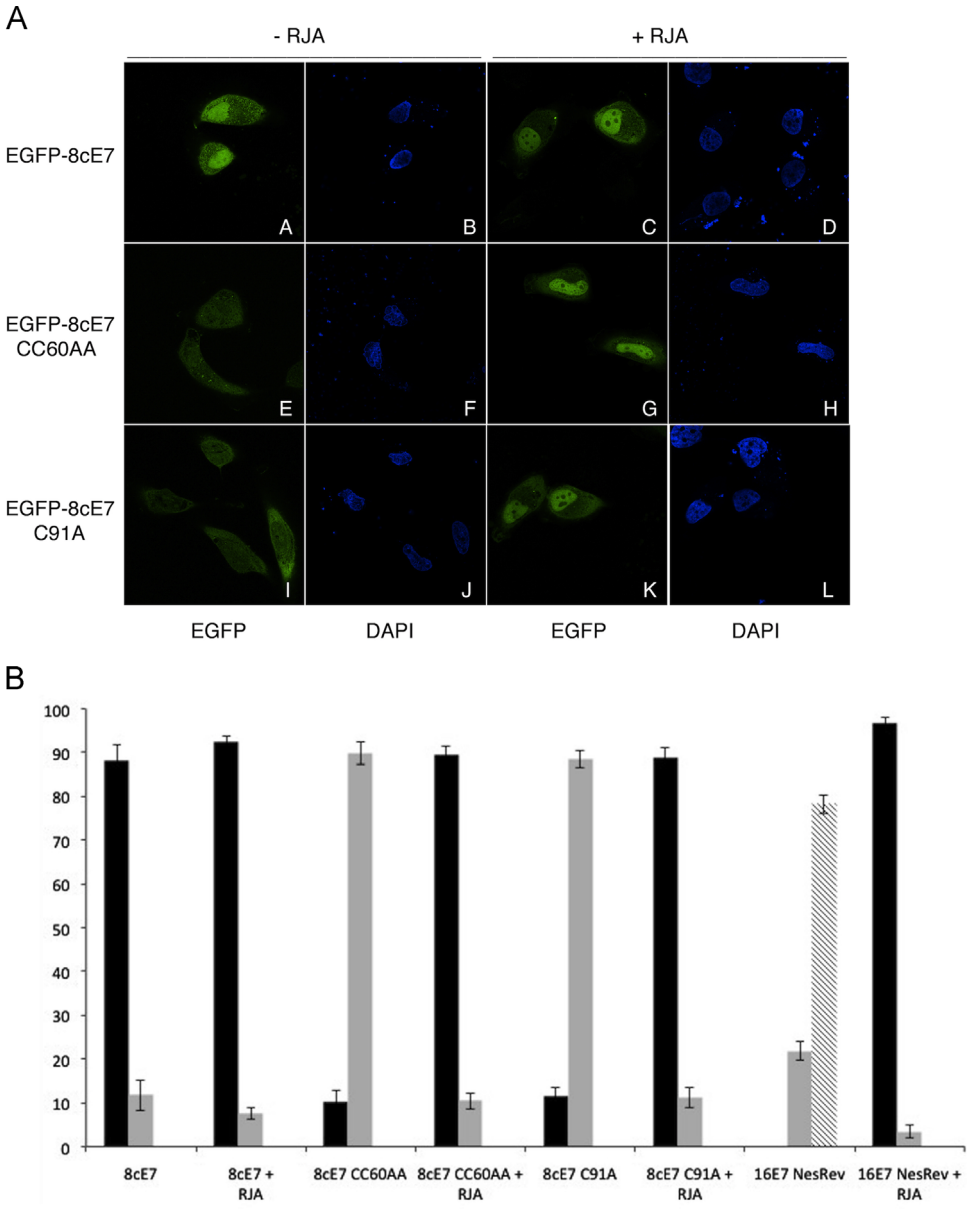

Fig. 1. (A) The pancellular localization of two EGFP–8cE7 cysteine mutants changes to nuclear in the presence of RJA, a specific inhibitor of CRM1 nuclear export receptor. Twenty hours post-transfection with EGFP–8cE7 (panels A, B, C and D), EGFP–8cE7 CC60AA (panels E, F, G and H) or EGFP–8cE7 C91A (panels I, J, K and L), HeLa cells were treated with either fresh DMEM (panels A, B, E, F, I and J) or 10 ng/mL RJA (panels C, D, G, H, K and L) for 4 h. After fixation, the cells were examined by confocal microscopy. Panels A, C, E, G, I and K represent the EGFP fluorescence and panels B, D, F, H, J and L represent the DAPI staining of the nuclei. (B) Quantitative analysis of the intracellular localization of EGFP–8cE7 and its cysteine mutants in the presence of RJA inhibitor. Twenty hours post-transfection with EGFP–8cE7, EGFP–8cE7 CC60AA, EGFP–8cE7 C91A or EGFP-16E7NesRev, HeLa cells were treated with either fresh DMEM or 10 ng/mL RJA for 4 h. After confocal microscopy analysis, cells were phenotyped as having either mostly nuclear (black bars), pancellular (gray bars) or mostly cytoplasmic (striped bars) and the quantitative analysis of five experiments is represented graphically showing the average values and standard deviations.

nuclear export. We have previously shown that the mucosal low risk HPV11 E7 has a leucine-rich NES ($_{76}$**I**RQ**L**QD**LLL**$_{84}$) within the zinc-binding domain that interacts with CRM1 (McKee et al., 2013). We also found that the zinc-binding domain of HPV16 E7 containing the leucine-rich NES interacts with CRM1 (data not shown). Another cutaneous beta genus HPV5 E7 protein has also a potential leucine-rich NES (**I**RA**F**QQ**LL**) within its zinc-binding domain that interacts with CRM1 export receptor (data not shown). This suggests that both the mucosal and cutaneous HPV E7 oncoproteins have evolved leucine-rich NESs that would mediate their nuclear export via a CRM1 pathway.

Overall, this work and a previous study (Onder and Moroianu, 2014) show that cutaneous HPV8 E7 shuttles between the nucleus and the cytoplasm and localizes predominantly in the nucleus with low levels in the cytoplasm, reflecting the contribution to the HPV8 E7 localization of the NLS and NES, respectively. The mucosal HPV16 E7 and HPV11 E7 oncoproteins also shuttle between the nucleus and the cytoplasm and this is in agreement with the fact that these HPV E7 oncoproteins have cellular targets in both cellular compartments.

3. Materials and methods

3.1. Mutagenesis to generate different EGFP-8E7 and EGFP–8cE7 mutants

The EGFP-8E7, EGFP–8cE7, EGFP–8cE7 CC60AA, EGFP–8cE7 C91A plasmids were previously generated (Onder and Moroianu,

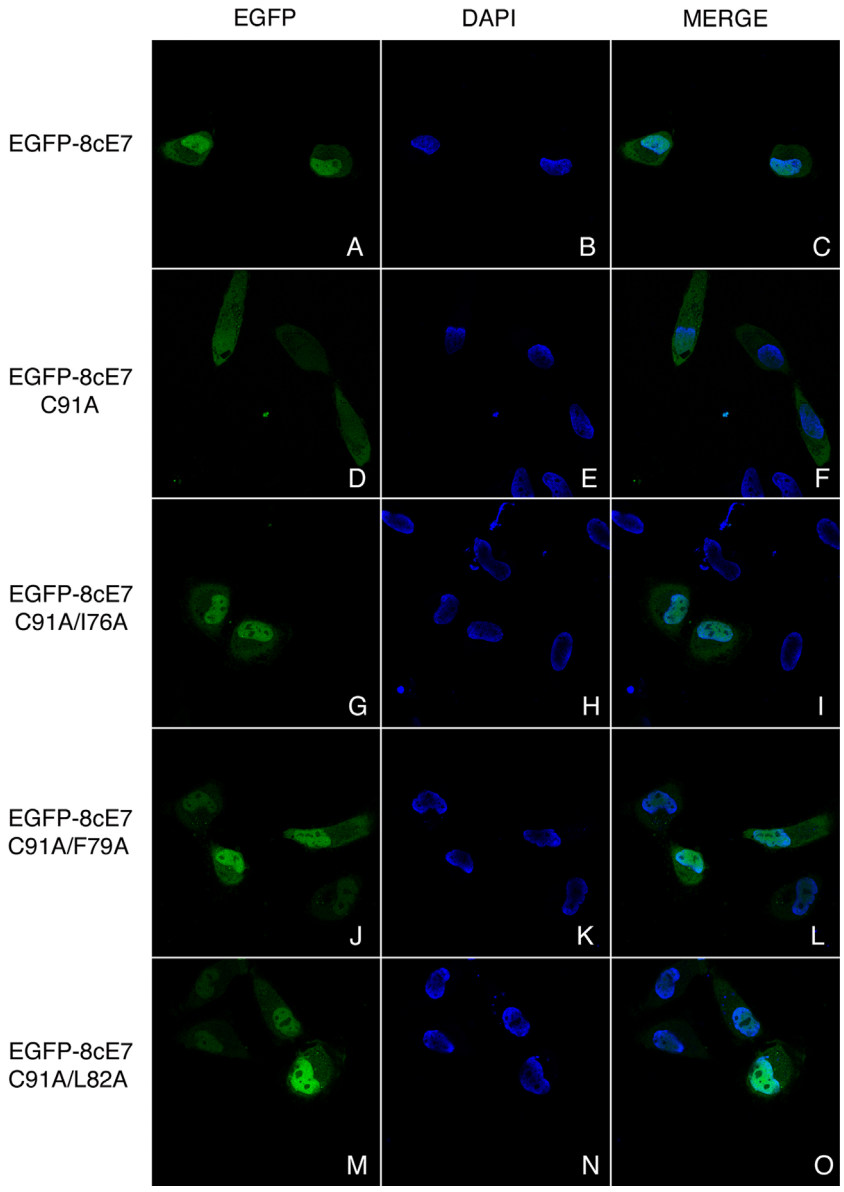

Fig. 2. Mutations in the potential NES change the localization of the EGFP–8cE7 C91A mutant from pancellular to nuclear. HeLa cells expressing either EGFP–8cE7 (panels A, B and C), EGFP–8cE7 C91A (panels D, E and F), EGFP–8cE7 C91A/I76A (panels G, H and I), EGFP–8cE7 C91A/F79A (panels J, K and L) and EGFP–8cE7 C91A/L82A (panels M, N and O) were fixed 24 h after transfection and examined by confocal microscopy. Panels A, D, G, J and M represent EGFP fluorescence, while panels B, E, H, K and N represent DAPI staining of the nuclei and panels C, F, I, L and O represent merge images.

2014). EGFP–8cE7$_{43-103}$ contains the zinc-binding domain of 8E7 (Onder and Moroianu, 2014). Site-directed mutagenesis was performed to generate EGFP–8cE7 C91A/I76A, EGFP–8cE7 C91A/F79A and EGFP–8cE7 C91A/L82A using the QuikChange Site-Directed Mutagenesis Kit (Stratagene) with EGFP–8cE7 C91A as template, and the corresponding mutagenesis primer pairs. All mutant plasmids were transformed into XL1-Blue competent cells (Agilent Technologies), purified and sequenced for verification (Eurofins MWG). The EGFP-16E7$_{NesRev}$ plasmid was previously generated (Knapp et al., 2009).

3.2. Transient transfections

Transient transfections were performed in HeLa cells (ATCC) as previously described (Onder and Moroianu, 2014). HeLa cells plated at 50–70% confluency on 12 mm poly-L-lysine coated glass coverslips 24 h before transfection were transiently transfected with the EGFP construct of interest (as indicated in the figure legends) using FuGENE 6 Transfection Reagent (Roche Applied Science, IN). The media was changed to DMEM with 10% FBS and 1% penicillin–streptomycin after 6 h and the cells were fixed with 3.7% formaldehyde in PBS for 10 min. Coverslips were mounted with Vectashield-DAPI mounting medium (Vector Labs, CA) to identify the nuclei of cells. Fixed cells were analyzed with a Leica TCS Sp5 broadband confocal microscope using a 63 × immersion oil objective and 1.6 × optical zoom and representative images were taken using Leica LAS AF software (Leica Microsystems). The intracellular localization of EGFP-tagged proteins was phenotyped as either mostly nuclear, pancellular (both in the cytoplasm and nucleus with no accumulation in either compartments), or mostly cytoplasmic and data from at least 4 experiments were quantitated and graphical representations were used to display average values and standard deviations.

3.3. Ratjadone A (RJA) treatment

Twenty hours post-transfection with EGFP fusion plasmids one set of HeLa cells were treated with 10 ng/mL RJA (Calbiochem) in

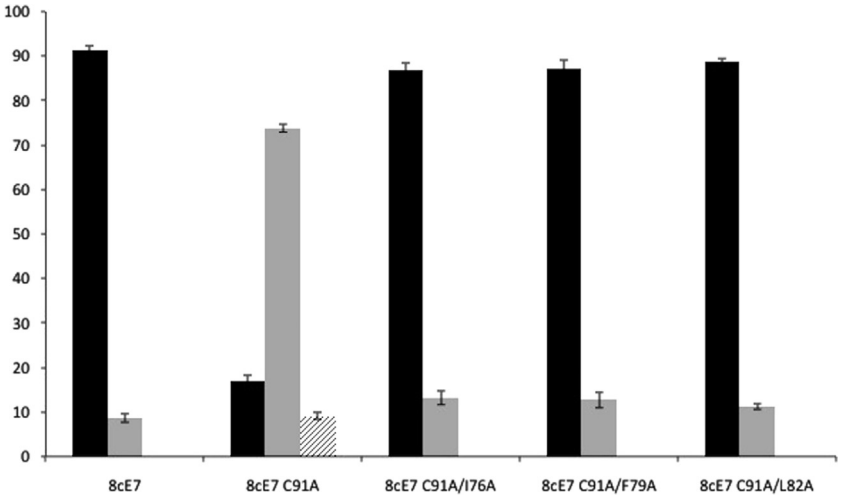

Fig. 3. Quantitative analysis of the effect of NES mutations on the localization of EGFP–8cE7 C91A mutant. Cells transfected with either EGFP–8cE7, EGFP–8cE7 C91A, EGFP–8cE7 C91A/I76A, EGFP–8cE7 C91A/F79A or EGFP–8cE7 C91A/L82A were phenotyped as having either mostly nuclear (black bars), pancellular (gray bars) or mostly cytoplasmic (striped bars) and the quantitative analysis of four experiments is represented graphically.

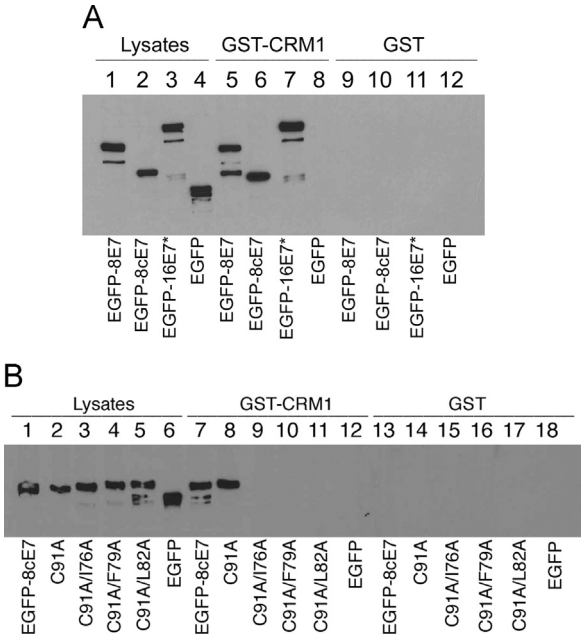

Fig. 4. (A) HPV8 E7 interacts with CRM1 nuclear export receptor. HeLa cell lysates were prepared 24 h post transfection with EGFP-8E7 (lane 1), EGFP–8cE7 (lane 2), EGFP-16E7⁎ (containing the NES of HIV Rev at the C-terminus) (lane 3) or EGFP (lane 4) and incubated with either GST-CRM1 or GST immobilized on glutathione-Sepharose beads. Both cell lysates (lanes 1–4) and the bound proteins that were eluted (lanes 5–12) were analyzed by immunoblotting with an anti-GFP antibody. (B) NES mutations in the context of EGFP–8cE7 C91A disrupt the interaction between 8cE7 and CRM1. HeLa cell lysates were prepared 24 h post transfection with EGFP–8cE7, EGFP–8cE7 C91A, EGFP–8cE7 C91A/I76A, EGFP–8cE7 C91A/F79A, EGFP–8cE7 C91A/L82A or EGFP, and incubated with either GST-CRM1 or GST immobilized on glutathione-Sepharose beads. Both cell lysates and the bound proteins that were eluted were analyzed by immunoblotting with an anti-GFP antibody (EGFP–8cE7, lanes 1, 7, 13; EGFP–8cE7 C91A, lanes 2, 8, 14; EGFP–8cE7 C91A/I76A, lanes 3, 9, 15; EGFP–8cE7 C91A/F79A, lanes 4, 10, 16 EGFP–8cE7 C91A/L82A, lanes 5, 11, 17; EGFP, lanes 6, 12, 18).

DMEM^{+} for 4 h in the dark, as previously described (McKee et al., 2013). After treatment, cells were fixed and analyzed by confocal microscopy as previously described. EGFP-16E7 tagged with the nuclear export signal of HIV-Rev (EGFP-16E7$_{NesRev}$) was used as a positive control for the RJA experiments.

3.4. Purification of GST fusion proteins

The GST-CRM1 plasmid was kindly provided by Dr. Jorgen Kjems. *Escherichia coli* BL21 CodonPlus cells (Agilent Technologies) previously transformed by either GST-CRM1 or GST plasmid were induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at 37 °C for 2 h. The proteins were then purified in their native state on glutathione-Sepharose beads (GE Healthcare) according to a standard purification procedure. The eluted proteins were checked for purity and lack of degradation by 12% SDS-PAGE and Coomassie Blue staining, and they were stored in aliquots at −80 °C until use.

3.5. In vitro isolation assays

Before performing the isolation assays, GST-CRM1 and GST immobilized on glutathione-Sepharose beads were analyzed by SDS-PAGE and Coomassie blue staining to ensure the proteins were intact and bound to beads at similar levels. Equal amounts of cellular lysates (in volume) containing either EGFP–8cE7, EGFP–8cE7 C91A, EGFP–8cE7 C91A/I76A, EGFP–8cE7 C91A/F79A, EGFP–8cE7 C91A/L82A or EGFP and 1 mM GTP-γS were incubated with either GST-CRM1 or GST immobilized on glutathione-Sepharose beads for 1 h at 4 °C. Cellular lysates and the eluted bound proteins were analyzed by immunoblotting with anti-GFP antibody (Clontech).

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References

Akgul, B., Cooke, J.C., Storey, A., 2006a. HPV-associated skin disease. J. Pathol. 208, 165–175.

Akgul, B., Garcia-Escudero, R., Ghali, L., Pfister, H.J., Fuchs, P.G., Navsaria, H., Storey, A., 2005. The E7 protein of cutaneous human papillomavirus type 8 causes invasion of human keratinocytes into the dermis in organotypic cultures of skin. Cancer Res. 65, 2216–2223.

Akgul, B., Ghali, L., Davies, D., Pfister, H., Leigh, I.M., Storey, A., 2007. HPV8 early genes modulate differentiation and cell cycle of primary human adult keratinocytes. Exp. Dermatol. 16, 590–599.

Akgul, B., Pfefferle, R., Marcuzzi, G.P., Zigrino, P., Krieg, T., Pfister, H., Mauch, C., 2006b. Expression of matrix metalloproteinase (MMP)-2, MMP-9, MMP-13, and MT1-MMP in skin tumors of human papillomavirus type 8 transgenic mice. Exp. Dermatol. 15, 35–42.

Angeline, M., Merle, E., Moroianu, J., 2003. The E7 oncoprotein of high-risk human papillomavirus type 16 enters the nucleus via a nonclassical Ran-dependent pathway. Virology 317, 13–23.

Bouwes Bavinck, J.N., Plasmeijer, E.I., Feltkamp, M.C., 2008. Beta-papillomavirus infection and skin cancer. J. Investig. Dermatol. 128, 1355–1358.

Dubina, M., Goldenberg, G., 2009. Viral-associated nonmelanoma skin cancers: a review. Am. J. Dermatopathol. 31, 561–573.

Eberhard, J., Onder, Z., Moroianu, J., 2013. Nuclear import of high risk HPV16 E7 oncoprotein is mediated by its zinc-binding domain via hydrophobic interactions with Nup62. Virology 446, 334–345.

Feltkamp, M.C., de Koning, M.N., Bavinck, J.N., Ter Schegget, J., 2008. Betapapillomaviruses: innocent bystanders or causes of skin cancer. J. Clin. Virol.: Off. Publ. Pan Am. Soc. Clin. Virol. 43, 353–360.

Hutten, S., Kehlenbach, R.H., 2007. CRM1-mediated nuclear export: to the pore and beyond. Trends Cell Biol. 17, 193–201.

Jones, D.L., Munger, K., 1996. Interactions of the human papillomavirus E7 protein with cell cycle regulators. Semin. Cancer Biol. 7, 327–337.

Knapp, A.A., McManus, P.M., Bockstall, K., Moroianu, J., 2009. Identification of the nuclear localization and export signals of high risk HPV16 E7 oncoprotein. Virology 383, 60–68.

McLaughlin-Drubin, M.E., Munger, K., 2009. The human papillomavirus E7 oncoprotein. Virology 384, 335–344.

McKee, C.H., Onder, Z., Ashok, A., Cardoso, R., Moroianu, J., 2013. Characterization of the transport signals that mediate the nucleocytoplasmic traffic of low risk HPV11 E7. Virology 443, 113–122.

Meissner, T., Krause, E., Vinkemeier, U., 2004. Ratjadone and leptomycin B block CRM1-dependent nuclear export by identical mechanisms. FEBS Lett. 576, 27–30.

Onder, Z., Moroianu, J., 2014. Nuclear import of cutaneous beta genus HPV8 E7 oncoprotein is mediated by hydrophobic interactions between its zinc-binding domain and FG nucleoporins. Virology 449, 150–162.

Piccioli, Z., McKee, C.H., Leszczynski, A., Onder, Z., Hannah, E.C., Mamoor, S., Crosby, L., Moroianu, J., 2010. The nuclear localization of low risk HPV11 E7 protein mediated by its zinc binding domain is independent of nuclear import receptors. Virology 407, 100–109.

Sperling, T., Oldak, M., Walch-Ruckheim, B., Wickenhauser, C., Doorbar, J., Pfister, H., Malejczyk, M., Majewski, S., Keates, A.C., Smola, S., 2012. Human papillomavirus type 8 interferes with a novel C/EBPbeta-mediated mechanism of keratinocyte CCL20 chemokine expression and Langerhans cell migration. PLoS Pathog. 8, e1002833.

Yamashita, T., Segawa, K., Fujinaga, Y., Nishikawa, T., Fujinaga, K., 1993. Biological and biochemical activity of E7 genes of the cutaneous human papillomavirus type 5 and 8. Oncogene 8, 2433–2441.

zur Hausen, H., 2009. Papillomaviruses in the causation of human cancers – a brief historical account. Virology 384, 260–265.

Zwerschke, W., Jansen-Durr, P., 2000. Cell transformation by the E7 oncoprotein of human papillomavirus type 16: interactions with nuclear and cytoplasmic target proteins. Adv. Cancer Res. 78, 1–29.