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# RNA splicing factors regulated by HPV16 during cervical tumour progression

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## Abstract

The most prevalent human papillomaviruses (HPVs) causing cervical disease are the “high risk” HPV types 16 and 18. All papillomaviruses express a transcription factor, E2 that can regulate viral and cellular gene expression. Recently, we demonstrated high risk HPV E2-mediated transcriptional transactivation of SF2/ASF. This essential oncoprotein is a key member of a family of proteins, the SR proteins that regulate constitutive and alternative splicing. Tight control of RNA splicing is necessary for production of wild type proteins. So, aberrant expression of SR proteins is involved in the aetiology of a range of human diseases, including cancer. Here we demonstrate epithelial differentiation-specific control of SF2/ASF in HPV16 infected keratinocytes in organotypic raft culture and in low grade cervical lesions (CIN1). Further, we demonstrate HPV16 infection/differentiation-induced up-regulation of a specific subset of SR proteins and present evidence that HPV16 E2 controls expression of SRp20, SC35 and SRp75. Using a series of cell lines that model cervical tumour progression we show SF2/ASF, SRp20 and SC35 are specifically up-regulated in a model of cervical tumour progression. These SR proteins are also overexpressed in high grade cervical lesions, indicating that they may all have oncogenic functions. SR proteins could be useful biomarkers for HPV-associated disease.

### Keywords

human papillomavirus type 16; cervix; CIN; immunohistochemistry; SR proteins; SF2/ASF; HPV16 E2

## Introduction

Persistent infection of cervical epithelial cells with “high risk” human papillomaviruses (HPV) can lead to premalignant and malignant cervical lesions [1]. HPV type 16 (HPV16) is the most prevalent anogenital infective HPV type causing over 50% of cases of cervical cancer. During infection, 50-100 episomal HPV genomes are present in the nuclei of infected cells [2]. With time, an immune response is mounted against infection and lesions regress [3]. However, sometimes virus genome(s) integrate into the host genome. Upon

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integration, most of the virus genome is lost [4] including the E2 viral transcription factor coding region [5] leading to high E6/E7 viral oncoprotein expression and cervical tumour progression. Viral genome integration results in complete disruption of the virus life cycle.

RNA splicing is the process of removal of non-coding introns from pre-mRNA and exon joining to form functional mRNAs. Correct and precise splicing is crucial for expression of wild type proteins. Many human diseases are associated with aberrations in splicing [6]. There are 8 “classical” serine-arginine-rich (SR) proteins [7;8] that regulate cellular and viral splicing. High risk HPV16 E2 (16E2) controls expression of the prototypical SR protein, SF2/ASF, that is expressed at high levels in HPV-infected, differentiated monolayer cervical epithelial cells [9;10]. Uninfected differentiated keratinocytes express only low levels of the protein [10;11] as they begin to shut down nuclear function [12]. However, virus late RNAs that encode the capsid proteins are present [13] and require extensive splicing in differentiated epithelial cells [14]. 16E2 may up-regulate SF2/ASF to facilitate late events in the virus life cycle.

We demonstrate that SF2/ASF is up-regulated in response to differentiation in HPV-infected cervical epithelial raft tissue and low grade cervical lesions. 16 E2 regulates a specific subset of SR proteins, SF2/ASF, SRp20 and SC35. These are also overexpressed, but by a different mechanism, in cervical tumour progression.

## Methods

### Cell culture

W12E (20863) and W12G (20861) were grown and differentiated as described [15]. W12GPX and W12GPXY were cultured as described [16]. HaCaT and U2OS cells were cultured in DMEM, 10% foetal calf serum, 2mM glutamine. HaCaT cells were differentiated as for W12E cells.

### Cervical lesions and HPV detection

Archival paraffin-embedded cervical biopsy samples and normal cervical tissue from hysterectomies were obtained with ethical permission (Glasgow Royal Infirmary). Diagnosis was made by a gynaecological pathologist. HPV presence was confirmed using the GP5+/GP6+ primer set in PCR. 10 HPV16-positive samples graded CIN 1 and 10 graded CIN 3, were studied, together with 8 normal tissues. HPV16 detection in 10 μm sections from paraffin-embedded samples was carried out using the Puregene DNA purification kit (Gentra systems) and PCR amplification of the L1 region using primers L1LBCfor 5′-CGAGCACAGGGCCACAATAATGGC-3′ (nts 6513-6539) and L1LBCrev 5′-GGAAACTGATCTAGGTCTGCAG-3′ (nts 7023-7003) and 35 cycles of 30 secs denaturation at 94°C, 30 secs annealing at 58 °C and 60 secs extension at 72 °C.

### Preparation of protein extracts

Cells were lysed in 2 × SDSPAGE loading buffer containing protease and phosphatase inhibitors (Roche Diagnostics). DNA was sheared through a 22 gauge needle 10 times. Sample volume for loading on gels was calculated according to cell numbers. Alternatively, keratinocytes were harvested by scraping into PBS and pelleted by centrifugation followed by freeze-thawing 3 times in 0.25 M Tris-HCl, pH 7.5 with protease and phosphatase inhibitors. Protein concentration was determined by Bradford assay (BioRad). Both protocols gave very similar results in western blotting.

### Western blotting

Proteins were separated on 12% polyacrylamide gels (midi BioRad Protean) and electroblotted onto PVDF membranes. Primary antibodies were diluted in PBS with 5% dried milk powder and 0.05% (v/v) Tween-20 (PBS-T). Mab104 was a hybridoma supernatant prepared from cell line ATCC: mAb104; SF2/ASF clone 96 and SRp20 clone7B4; Zymed Laboratories; 9G8, clone 98; gift of Dr James Stevenin, Strasbourg, GAPDH clone 6CS; Biodesign International and involucrin antibody SY5: Sigma. E2 antibody was TVG261.

### Organotypic raft culture

Raft culture was carried out as described [17]. $1 \times 10^6$ epithelial cells in E-medium were applied to collagen rafts and incubated until a monolayer formed then lifted to the air-liquid interface on a wire grid. Rafts were grown for 14 days, fixed in 10% neutral-buffered formalin overnight, paraffin-embedded, cut into 4 μm cross sections and placed on poly-L-lysine (Sigma) slides.

### Immunohistochemistry

Staining was performed in the laboratory using the ABC Elite kit (Vector Laboratories) or by the Institute of Comparative Medicine's Histopathology service. Sections were dewaxed in xylene and rehydrated through a graded series of alcohol (from 100% to 50%). Antigen retrieval was by microwaving in citrate buffer (1.5 mM citric acid, 8.0 mM Tris-sodium citrate) for 15 min before blocking with normal blocking buffer (Vectastain kit) for 30 min except for cytokeratins 10 and 14 where sections were treated with Proteinase K (Dako). Endogenous peroxidase was blocked (Envision Kit Dako Cytomation). Antibodies were added following blocking in PBS-20% horse serum and incubated for 2 h at 4°C. Slides were washed in Tris-Tween before application of the secondary antibody conjugated with horse radish peroxidise for 30 min. Sections were washed as above and stained with DAB chromagen and counterstained with haematoxylin. Control reactions in the absence of primary antibody showed no staining (data not shown).

## Results

### Levels of SF2/ASF increase in the suprabasal layers of HPV16-infected cervical epithelia

W12E cells, from a patient with a low grade cervical lesion (CIN 1), contain around 100 episomal HPV16 genomes, the infective form of the virus [18]. These basal epithelial cells can differentiate in monolayer culture to express keratinocyte differentiation markers [10;14;19], high levels of virus late mRNAs [10;14] and abundant SF2/ASF [10]. SF2/ASF expression patterns were examined in W12E raft tissue sections (clone 20863, [15]) and compared to raft sections of W12G cells (clone 20861, [15]) that contain integrated HPV16 genomes, a dead-end for virus infection, and HaCaT cells, keratinocytes that do not contain HPV16 genomes [20]. Figure 1A shows strong staining in the W12E nuclei in the mid to upper epithelial layers. Staining in the W12G raft section was weak with no distinct pattern (Figure 1B). In contrast, SF2/ASF was detected mainly in the basal layer nuclei of HaCaT rafts (Figure 1C). These observations were consistently observed in at least three different raft cultures for each cell line.

SF2/ASF expression was also analysed in a series of HPV16-positive CIN 1 lesions. Figure 2A shows a representative lesion with strong but mosaic SF2/ASF staining in the mid to upper epithelial layers. There was reduced staining in the superficial layers. Figure 2B shows adjacent epithelium from the same tissue section with more normal morphology. Here staining was weak, mainly in the lower epithelial layers. Figure 2C shows a different CIN 1 lesion with a "normal" section adjacent to a region of low grade disease highlighting the

difference between the two staining patterns. Haematoxylin and eosin staining demonstrated abnormal mitoses and koilocytic cells in diseased areas of tissues (data not shown).

Staining of CIN 3 lesions revealed high levels of SF2/ASF expression throughout the epithelium (Figure 2D). The pattern in Figure 2D differs from Figure 2A in that the staining was more uniform throughout the epithelial layers and was detected even in the outermost layer (arrow). A “normal” section of epithelium from the same tissue section is shown for comparison (Figure 2E). This region of the epithelium appears thicker because the section has been cross-cut at this point. The pattern observed here is quite different to that shown in Figure 2D but similar to that in Figure 2B. A transition in a section between CIN3 and a more “normal” section was not found.

We noted three different SF2/ASF staining patterns (Figure 3A). Pattern 1 has staining mostly in the basal layer cells. Pattern 3 has staining in all cells through all layers. Pattern 2 is intermediate with staining of the basal layer plus a subset of cells in the suprabasal layers (see Figure 7). Staining does not reach the outermost epithelial layer. The table in Figure 3B indicates that most low grade cervical lesions showed pattern 2 while high grade lesions showed pattern 3. All normal tissues except one showed staining pattern 1. The tissue that displayed pattern 2 was positive for HPV16 and a low grade lesion was likely missed in this case. Two other normal tissues were HPV-positive but did not display pattern 2. The viral load in these cases may have been less that in the tissue that displayed pattern 2 leading to reduced induction of SF2/ASF in the suprabasal epithelial layers. Some CIN tissues had areas that displayed less progressed morphology, for example epithelium with characteristics of CIN1 was sometimes observed in one part of the section with a normal piece of epithelium present in another (e.g. Figure 2C). So a mixture of patterns was observed in some cases. Staining with Ki67, cytokeratin 10 and cytokeratin 14 antibodies revealed expected levels of division and differentiation in the tissues (data not shown).

## Bimodal expression of SF2/ASF during cervical tumour progression

We examined SF2/ASF expression in a W12-derived model of cervical tumour progression [16] (Figure 4A). W12E and G cells are immortal but not transformed. Figure 4B shows abundant SF2/ASF in W12E cells (lane 1) expressing16E2 that controls SF2/ASF levels [9;10] but reduced SF2/ASF in W12 G cells that do not express 16E2 (lane 2). The two transformed cell lines, W12GPX (lane 3) and W12GPXY (lane 4) displayed significantly higher levels of SF2/ASF than W12G (Figure 4D), from which they were derived sequentially, and also did not express 16E2 (Figure 4B). This indicates that cervical tumour progression can result in increased levels of SF2/ASF by means other than 16E2 regulation. SF2/ASF levels were compared to the functionally related SRp20 and 9G8 SR proteins in the W12 lines. SRp20 expression levels followed the pattern of SF2/ASF (Figure 4B and E) but 9G8 did not: there was a similar level of 9G8 in all the lines (Figure 4B and C). Commercially available antibodies against other classical SR proteins are not efficient in western blotting and immunohistochemistry or react against multiple SR proteins. However, SC35 antibody works well in immunohistochemistry, as does the SRp20 antibody and both proteins are abundant throughout CIN 3 lesions (Figure 4F). The 9G8 antibody does not work in immunohistochemistry.

## A subset of SR proteins display elevated levels in W12E cells

To determine whether other SR proteins are regulated by HPV16 infection, W12E (episomal virus genomes) and HaCaT (no virus genomes) cells were grown in monolayer culture to give undifferentiated and differentiated cell populations. SR protein levels were determined by semi-quantitative western blotting with Mab104 that detects the majority of classical SR proteins and with specific monoclonal antibodies against SRp20 and 9G8 that are not

efficiently detected by Mab104. SF2/ASF, SC35 and SRp30C migrate together in SDSPAGE (SRp30s, bracketed, Figure 5). The lower band is SC35, the upper band SF2/ASF, while SRp30C is not detected by Mab104. A separate western blot probed with Mab96 against SF2/ASF is also shown for comparison. Differentiation of the cell populations was indicated by an increase in involucrin expression. In HaCaT cells (Figure 5A) SR proteins levels remained the same (e.g. SRp40) or decreased slightly (SRp55) upon epithelial differentiation. In contrast, levels of SF2/ASF increased upon W12E cell differentiation (Figure 5B) as did SRp20 and SC35. SRp40 and SRp55 levels also increased but to a lesser extent. SRp75 could not be detected because it is expressed at reduced levels in epithelial cells [21]. 9G8 levels did not change upon differentiation of W12E cells. SRp20 and SC35 expression increased in the mid to upper layers of HPV-positive CIN 1 lesions (Figure 5C).

### HPV16 E2 controls expression of a subset of SR proteins

To discover whether other SR proteins might be under 16E2 regulation SR protein levels were examined in two U2OS cell clones stably expressing 16E2 and in U2OS cells stably transfected with vector alone [22]. U2OS cells are p53-negative and can tolerate expression of HPV16 E2 that may trigger apoptosis [23]. Figure 6 shows that E2-expressing clone B1 showed increased levels of SF2/ASF (upper band, SRp30s) and also SRp20, SC35 (lower band, SRp30s) and SRp75. Clone A4 cells did not overexpress SF2/ASF but levels of SRp75, SC35 and SRp20 were increased over background levels in the control cells. U2OS are osteosarcoma cells. Although these cells are transformed, it is clear that, unlike the tumour progressed W12 cells, they express low levels of the SR proteins and respond to expression of 16E2. The differences detected in SR protein levels between the two cloned U2OS lines examined may be due to the different concentrations of E2 expressed in the cloned lines.

## Discussion

RNA splicing determines cellular protein expression, so defects in splicing lead to disease [6]. HPV gene expression is also regulated at the level of RNA splicing [14;24;25]. Splicing is controlled by SR proteins. Nuclear levels of the prototypical SR protein SF2/ASF are low in differentiated suprabasal epithelial cells [10;11] but abundant virus late RNAs that require splicing [14] are synthesised in these cells [13;26]. To assess SF2/ASF levels during HPV infection, previously we examined W12E cells (or CIN612 cells containing HPV31 episomal genomes [27]) differentiated in monolayer culture. However, although the cell populations contained differentiated cells, not all cells were fully differentiated (75-85% express involucrin, and cytokeratin 10, around 80% express the first virus late protein E1^E4, 5-10 % express filaggrin and the L1 capsid protein). So we examined the SF2/ASF expression pattern in three dimensional, fully differentiated tissues: W12E organotypic raft culture and CIN1 lesions. SF2/ASF expression was increased in the mid to upper epithelial layers of the tissues coinciding with the expression of 16E2 detected in koilocytes in suprabasal and superficial epithelial layers [28;29]. SF2/ASF may be transcriptionally up-regulated upon differentiation of HPV infected keratinocytes [11] by high risk HPV E2 to allow efficient processing of virus capsid-encoding RNAs.

A recent study reported that SF2/ASF was not upregulated in differentiated epithelial cells in CIN 1 lesions [11]. We have observed a consistent pattern of expression of SF2/ASF in five CIN 1 tissues where there is a transition in the epithelium from “normal” to CIN (e.g. Figure 2C) giving confidence in the staining patterns we report. A rather different pattern was observed in CIN 3 lesions. Here stained nuclei could be seen throughout the epithelium in a more uniform pattern.

Figure 7 shows a diagram of the three staining patterns we observed. In normal epithelium (Figure 7A), only the mitotically active basal layer cells express SF2/ASF. As epithelial cells differentiate they stop dividing and begin to shut down nuclear function [12] and this may be why less SF2/ASF is expressed in the upper layers of uninfected epithelium. In contrast, in cervical lesions with high risk HPV infection, cells in the mid to upper epithelial layer can re-enter the cell cycle. This alone should stimulate SF2/ASF production but production of HPVE2 may also result in increased expression in some cells. This may be why there is a mosaic pattern of staining in low grade cervical lesions (Figure 7B). This pattern reflects the sort of histological variation observed in any given lesion. Finally, in high grade lesions, mitotically active, transformed basal epithelial cells expressing high levels of SF2/ASF fill the epithelium (Figure 7C).

## SRp20 and SC35 are upregulated by HPV16E2

Similar to SF2/ASF, there was a significant increase in SRp20 and SC35 levels upon differentiation of W12E but not HaCaT cells indicating that HPV infection may regulate expression of this specific SR protein subset. Confirming this observation, SRp20 and SC35 gave a similar pattern of staining to that observed for SF2/ASF in CIN1 lesions with strong staining in the nuclei of the mid to upper epithelial layers. A very similar SRp20 staining pattern in CIN 1 lesions has recently been reported by another group [30]. The data indicate that SF2/ASF, SRp20 and SC35 expression is coincident with production of L1-encoding mRNAs that are present and spliced in the cell layers beneath those where L1 protein is produced [13]. Western blot analysis of cell extracts of U2OS cells stably expressing 16E2 demonstrated that SRp20, SC35, SRp75 and SF2/ASF could be regulated by 16E2. Interestingly, these SR proteins are expressed in suprabasal epithelial cells coincident with the reported site of 16E2 expression [28;29]. They may be important regulators of viral gene expression because they are controlled by virus infection. For example, they could select splice isoforms of virus RNAs, thus modulating the viral proteome, or they could regulate virus mRNA stability or translation [7]. Thus these factors are potential targets for antiviral therapy.

## A specific subset of SR proteins is upregulated in cervical cancer progression

SF2/ASF and SRp20 were readily detected in W12E cells that express 16E2 from episomal genomes. In contrast, levels were lower in W12G cells where 16E2 is not expressed. W12G cells contain integrated HPV16 genomes but are not transformed. Fully transformed and invasive W12GPX and W12GPXY cells, derived from W12G cells, also do not express 16E2 but levels of SRp20 and SF2/ASF were at least as high as in W12E cells. Other mechanisms related to the tumourigenic process, for example, amplification of the SFRS1 gene encoding SF2/ASF might cause this effect. SRp20, SF2/ASF and SC35 were overexpressed in CIN 3 lesions. These SR proteins are also overexpressed in ovarian tumours [31;32] and breast tumours [33] but not other tumour types [34] revealing possible tumour tissue-specific differences in SR overexpression. Increased abundance of SRp20 and SC35 might suggest a role in tumour formation. However, to date clear evidence of full oncogenic function has been demonstrated only for SF2/ASF that regulates alternative splicing of RNAs encoding growth and apoptosis control proteins [30;35]. Increased levels of SF2/ASF in HPV-infected epithelia may predispose cells to become transformed. Further, chromosomal changes leading to overexpression of the protein may contribute to cervical tumour progression.

The types of changes observed with SR proteins indicate that they may be useful markers for diagnosis of cervical tumour progression. High levels of the proteins mark the nuclei of rapidly dividing basal epithelial cells that fill the full thickness of the epithelium in high grade cervical lesions giving a uniform staining of cell nuclei throughout the layers. Low

grade lesions show a different pattern with strong but mosaic staining of nuclei in the mid to upper epithelial layers with little staining detected in the uppermost epithelial layers. Assessment of usefulness of these proteins as biomarkers requires further study in liquid based cytology samples. Superficial epithelial cells are collected in liquid based cytology. The prediction would be that high grade cervical lesion samples may show higher levels of these SR proteins than samples of low grade lesions.

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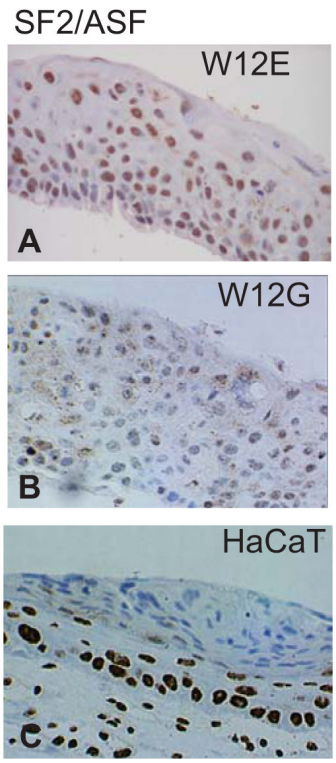


**Figure 1. SF2/ASF is expressed at high levels in the suprabasal layers of W12 cervical epithelial**

Immunohistochemical staining of sections of organotypic raft cultures of A. W12E cells (episomal HPV16 genomes), B. W12G cells (integrated HPV16 genomes) and C. HaCaT cells (no virus genomes) with Mab 96 against SF2/ASF. At least three raft sections for each cell line were grown and stained and representative images are shown. In C., HaCaT rafts, the collagen layer is present beneath the raft and fibroblasts in the collagen also stain with the SF2/ASF antibody. The collagen layers have been lost from the W12E and G raft tissues during paraffin-embedding. Sections were counterstained with haematoxylin.

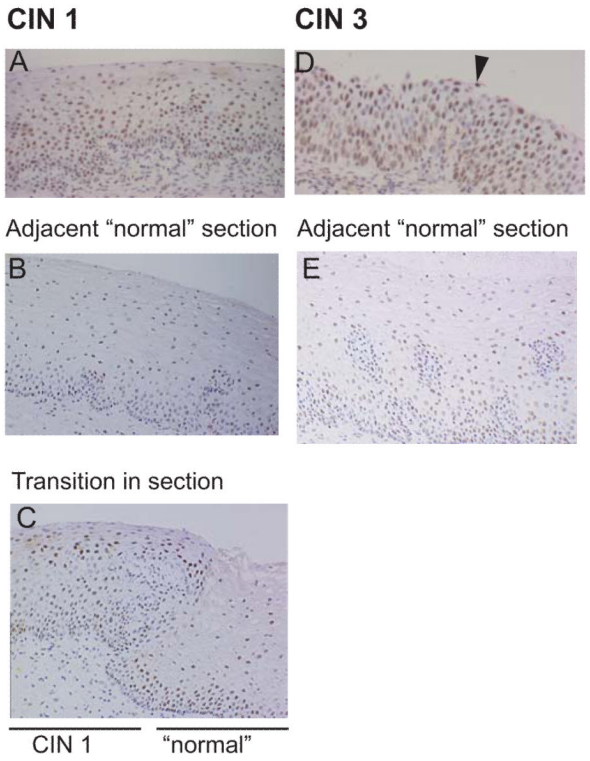


**Figure 2. Expression patterns of SF2/ASF in low and high grade cervical lesions**

Immunohistochemical staining of sections of paraffin-embedded cervical tissues with Mab 96 against SF2/ASF. A, B, C: CIN 1. A and B are from the same patient tissue section. C is from a different CIN 1 tissue and shows an area where a staining pattern similar to that in A is seen adjacent to a “normal” section of epithelium. D, E: CIN3. D and E are from the same patient tissue section. The arrowhead in D shows staining in cells in the outermost epithelial layer. Sections were counterstained with haematoxylin.




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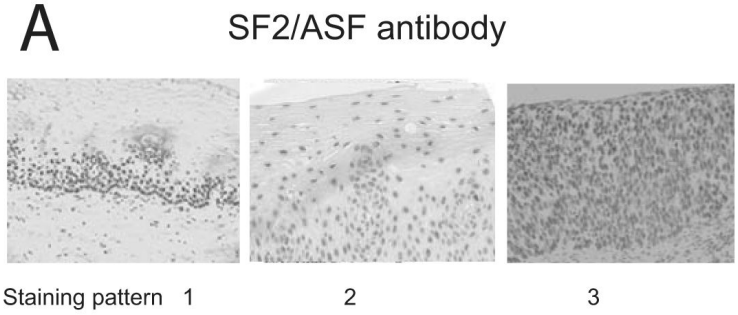
A




B

| Stage | No. of samples | HPV+ve | Staining pattern 1 | 2 | 3 |
|---|---|---|---|---|---|
| Normal | 8 | 3/8 | 7 | 1 | 0 |
| CIN 1 | 10 | 8/8 | 3 | 10 | 0 |
| CIN 3 | 10 | 10/10 | 4 | 5 | 10 |

**Figure 3. Summary of staining patterns obtained with a set of cervical tissues positive for HPV16 and graded as normal, CIN1 or CIN 3**

A. Representative SF2/ASF staining patterns from paraffin-embedded cervical samples. 1. Normal cervical epithelium. 2. Cervical epithelium with low grade disease features (CIN 1). 3. Cervical epithelium with high grade disease features (CIN 3). These are different tissues from those shown in Figure 2. B. Table showing distribution of staining patterns in a series of normal cervical epithelia and graded cervical lesions.

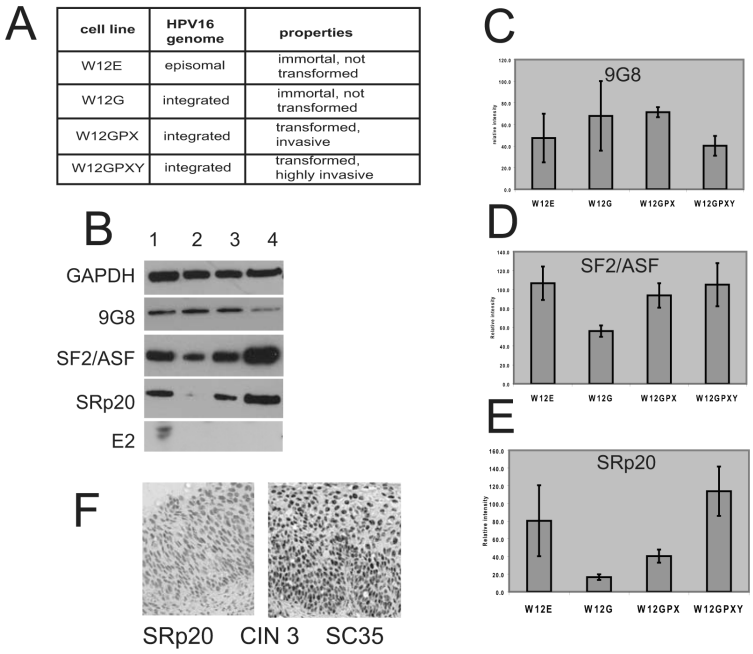



**Figure 4. SF2/ASF and SRp20 but not 9G8 are overexpressed in a model of cervical tumour progression**

A. Table describing the main properties of the four W12-derived cell lines used in this study [16]. B. Western blot analysis of levels of selected SR proteins and HPV16 E2 transcription factor in each of the W12 lines. 1. W12E, 2. W12G, 3. W12GPX, 4. W12GPXY. Each experiment was carried out at least three times. Band intensities were quantified using Image J relative to levels of GAPDH. The specificity of the E2 antibody TVG 216 is demonstrated in Figure 6. Graphs in C, D and E show the relative levels of the three SR proteins analysed in each cell line. F. Immunohistochemical staining of cervical lesions graded CIN 3 with SC35 and SRp20 antibodies.

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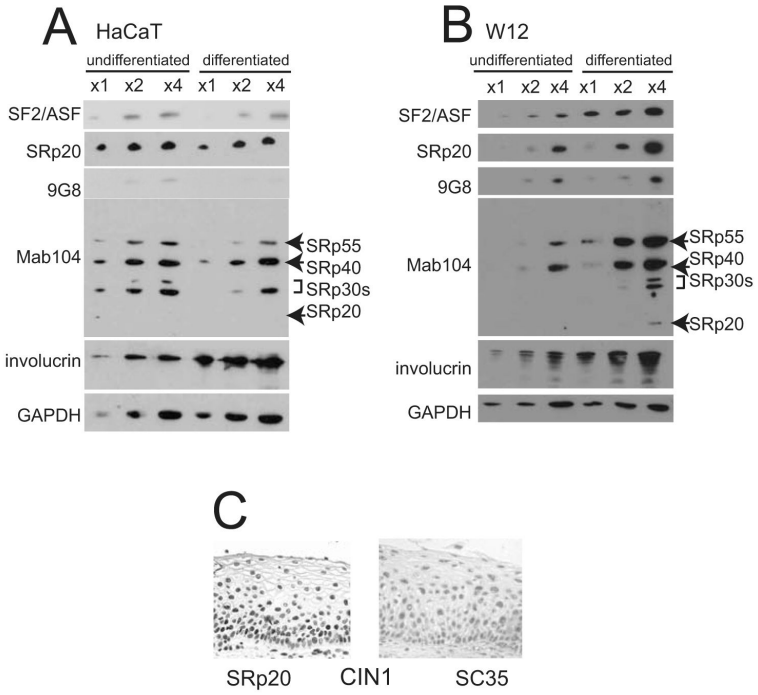


**Figure 5. A subset of SR proteins is up-regulated in response to epithelial differentiation**

Semi-quantitative western blot analysis of levels of the classical SR proteins in A. HaCaT cells (no virus genomes) and B. W12E cells (HPV16 episomal genomes). In each case cell populations that were 75-85% undifferentiated and 75-85% differentiated were generated by adjusting the density of the cells in culture and concentrations of $Ca^{++}$ in the grown media. x1, x2, and x4 refer to quantity of protein extracts added in each track where x1 is 5 μg. Antibodies used to probe each western blot strip are shown to the left hand side. Designations of SR proteins detected by Mab 104 are shown to the right hand side. SRp30s (bracketed) are lower band: SC35 and upper band: SF2/ASF. SRp30c is not detected by this antibody. Mabs104 and 96 detect phosphoepitopes so cells were lysed in SDS boiling buffer containing a range of protease and phosphatase inhibitors to inhibit degradation and dephosphorylation of the proteins that would alter their apparent abundance in western blotting. Mab104 blots were reprobed with GAPDH to show levels of protein loading in each track. The same samples were electrophoresed on identical gels, blotted and probed with an involucrin antibody to give an indication of differentiation of the cells. C. Paraffin-embedded cervical lesions graded CIN 1 were subject to immunohistochemistry using antibodies against SRp20 and SC35 to confirm up-regulation of these proteins in the differentiated layers of low grade cervical lesions (CIN 1).

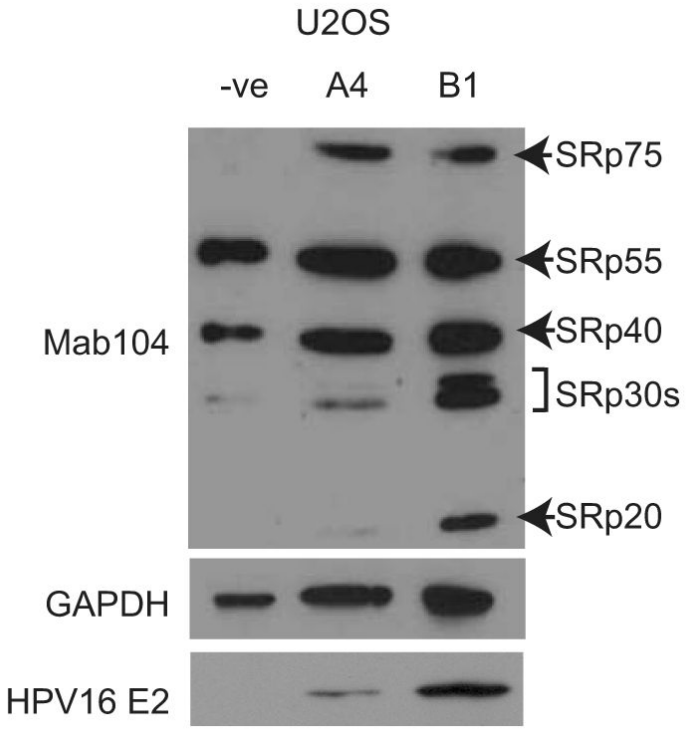


**Figure 6. HPV16 E2 can control levels of a subset of SR proteins in U2OS cells**

Western blot analysis of protein lysates of two clones (A4 and B1) [22] of U2OS osteosarcoma cells stably expressing HPV16 E2 and U2OS cells stably transfected with pCIneo vector alone (−ve) with Mab 104 that detects most classical SR proteins. Mabs104 and 96 detect phosphoepitopes so cells were lysed in SDS boiling buffer containing a range of protease and phosphatase inhibitors to inhibit degradation and dephosphorylation of the proteins that would alter their apparent abundance in western blotting. The experiment was repeated three times with very similar results. The blot was stripped and reprobed with GAPDH antibody as a loading control. A separate blot using the same amount of the same proteins lysates was probed with TVG261 antibody against HPV16 E2 to demonstrate expression of this transcription factor in the stably transfected U2OS cells.

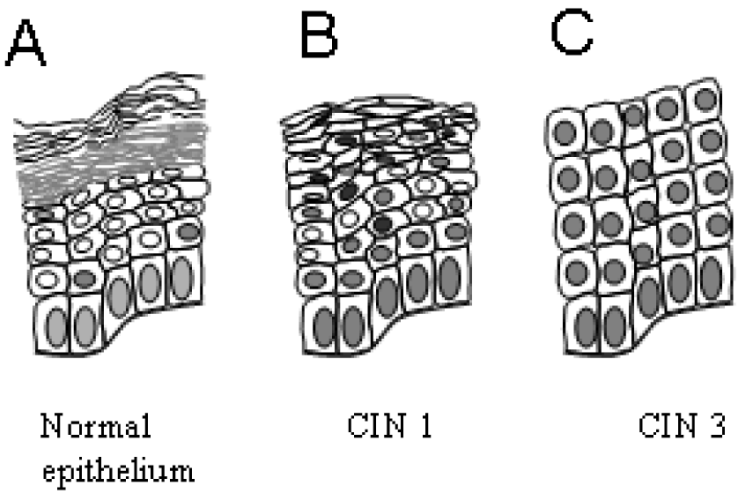


**Figure 7. A model of distribution of SF2/ASF in the epithelial layers of normal tissue and lesions graded CIN 1 and CIN 3**

A. Normal epithelium with well defined basal, suprabasal and granular layers. B. CIN 1 epithelium where the suprabasal compartment is expanded but cells still differentiate. C. CIN 3 epithelium where basal epithelial cells have filled most of the epithelium. Grey to black shading in the nuclei indicates presence of SF2/ASF.