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Importin-β and exportin-5 are indicators of acute viral infection: Correlation of their detection with commercially available detection kits

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ABSTRACT

This work focused on immunohistochemistry markers of acute viral infections. Viral infected cells were detected by in situ based methods (reovirus, rabies virus) or cytologic changes (human papillomavirus, molloscum contagiosum virus, herpes simplex virus). Two proteins involved in nuclear trafficking, importin-β and exportin-5, were detected in the infected cells for each virus and not in the control tissues. A wide variety of other proteins, including caspase-3, and bcl-2 family members (bcl2, bclX, MCL1, BAK, BAX, BIM, BAD) showed wide variations in expression among the different viral infections. Specificity of the importin-β and exportin-5 signals varied greatly with different commercially available peroxidase conjugates. It is concluded that immunohistochemistry detection of importin-β and exportin-5 may be useful markers of acute viral infection, which suggests that increased nuclear trafficking may be an important concomitant of viral proliferation.

1. Introduction

Viruses can be divided into those with double stranded DNA (6 families), single stranded RNA (14 families equally divided between sense and antisense RNA), singled stranded DNA (parvovirus), double stranded RNA (reovirus), and retroviruses [1-3]. Only a few members of these different viral families can induce cytologic changes diagnostic of that specific virus. Thus, the surgical pathologist usually relies on the in situ detection of a specific viral protein or RNA/DNA for a definitive diagnosis [3].

Viral proliferation in a given cell triggers both an immediate (innate) and delayed (adaptive) immune response [4-7]. Proteins such as RIG1 and multiple membrane bound toll like receptors (TLRs) initiate the innate immune system when the cell recognizes foreign DNA, RNA, or proteins. These initiating molecules, in turn, induce the expression of bridging proteins such as IRF3, MAVS, and NFK kappa β, which allow the express of key antiviral proteins such as interferon α, β and other cytokines such as IL-6 and TNF α [1,2,5-7]. Interferons, so named for their ability to interfere with viral infections, in turn induce a wide range of proteins referred to as the interferon stimulated genes, that reduce overall cell protein and RNA synthesis, induce caspase-3 mediated apoptosis, and increase MHC expression to prime the cell for cytotoxic T cell destruction [1,2,5-7]. Not surprisingly, many viruses have evolved mechanisms to inhibit the different arms of the innate immune response [1,2,6].

Importin-β and exportin-5 are proteins involved in nuclear trafficking that is an important mechanism whereby cells sequester proteins in compartments which either activate or inactivate them [8-11]. The HIV-1 protein Rev. can bind to both importin-β and some exportins which plays an essential role in viral infection since, in the absence of Rev., late structural mRNAs of HIV-1 are unable to leave the nucleus with reduced infectivity [12]. Drugs that can abrogate nuclear trafficking in viral infections such as ivermectin have been shown to block nuclear transport of, for example, the dengue viral NS5 protein that, in turn, markedly reduces viral infectivity [13]. Nonetheless, few in situ based studies have analyzed the expression of importin-β or exportin-5 in viral infections.

The purpose of this study was to examine the expression of importin-β and exportin-5 in a variety of RNA and DNA viral infections and to correlate this with multiple other proteins involved in the regulation of apoptosis.

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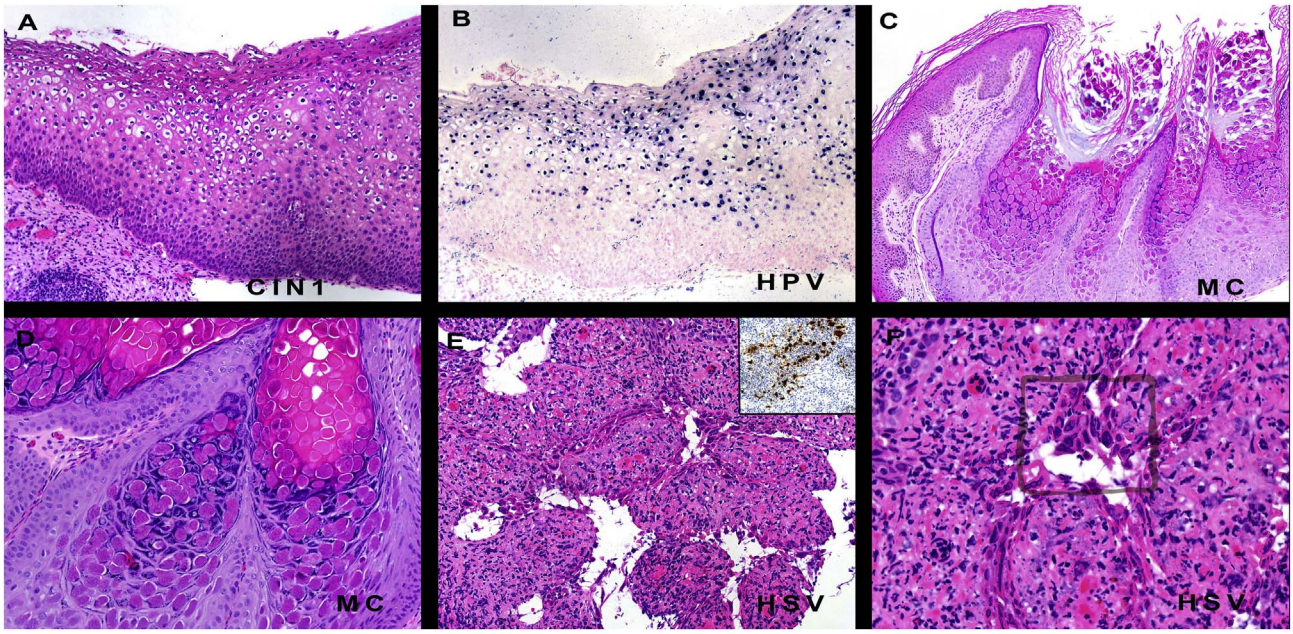

Fig. 1. Correlation of viral infection with histologic changes for three DNA viruses. Panel A shows the increased cell density, variable sized perinuclear halos, and variability in nuclear size, shape, and chromaticity diagnostic of a CIN 1 lesion. Note that the viral DNA is most abundant towards the surface of the lesion as demonstrated by in situ hybridization (panel B). Panel C shows a low magnification for a vulvar molloscum lesion; the large eosinophilic inclusions are evident at high magnification (panel D). Panels E and F are low and high magnifications of acute herpes simplex virus of the vulva. Note the marked inflammatory cell infiltrate and multinucleate cells with ground-glass changes (panel F, box). The insert in panel E shows the strong in situ hybridization signal for herpes simplex virus DNA.

2. Materials and methods

2.1. Tissues

Formalin fixed, paraffin embedded tissues were available from cervical or vulvar lesions with the diagnosis of cervical intraepithelial neoplasia (CIN 1) (10 cases), herpes simplex virus (10 cases), molloscum contagiosum (10 cases), and from humans infected brain tissues with rabies virus (2 cases with 2 controls), or mice infected with acute reovirus infection (3 cases with 3 controls). The rabies virus and reovirus infected tissues were previously reported [7, 14-16].

The histologic diagnosis of CIN 1, herpes simplex virus and molloscum contagiosum was made in part by the classic cytologic changes in the serial sections used for immunohistochemistry analysis. These included variable sized perinuclear halos, non-uniform nuclear size/shape/chromaticity, with irregular cell density (CIN 1), multinucleated squamous cells with ground glass nuclei (herpes simplex virus) and large, eosinophilic cytoplasmic inclusions (molloscum contagiosum). No histologic feature differentiated the reoviral or rabies virus infected tissues from the controls except for rare Negri bodies in the latter.

2.2. In situ hybridization

Our in situ hybridization protocol has been previously published [17-19]. In brief, in situ hybridization for human papillomavirus (HPV) DNA and herpes simplex virus DNA was done using the biotin tagged Pathogene probes from Enzo Life Sciences where the key steps included pretreatment in proteinase K, co-denaturation at 95C for 5 min, hybridization at 37C for 15 h, a stringent wash at 50C for 10 min, and precipitation of the 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (NBT/BCIP) chromogen due to the action of the alkaline phosphatase conjugated to streptavidin. Reoviral RNA was detected with the LNA modified oligomers that were 5′ tagged with digoxigenin as previously described; NBT/BCIP precipitation was induced by alkaline phosphatase conjugated to anti-digoxigenin [17-19]. Rabies virus RNA was detected by RT in situ PCR as well as LNA modified probes with equivalent results [14, 15].

2.3. Immunohistochemistry

Our immunohistochemistry protocol has been previously published [17-19]. The tissues were tested for the following antigens: importin-β, exportin-5, activated caspase-3, BAD, BIM (ABCAM), MCL1, bcl2, BAK, BAX, and bclX (Enzo Life Sciences) as well as, where relevant, rabies virus protein and reoviral capsid protein (kindly provided as a gift by Drs. Matt Coffey and Hue Tran). The analyses were done on the automated Leica Bond platform with the modification that we used the Enzo Life Sciences peroxidase conjugate (catalog # ADI-950-113-0100).

2.4. Co-expression analysis

Co-expression analyses were done using the Nuance system (CRI) as previously published [18,19]. In brief, a given tissue was tested for two different antigens using fast red and DAB as the chromogens. The results were then analyzed by the Nuance and inform systems in which each chromogenic signal is separated, converted to a fluorescence based signal, then mixed to determine what percentage of cells were expressing the two proteins of interest.

3. Results

3.1. DNA viruses: Correlation of the histologic changes with productive viral infection

The hematoxylin and eosin stains of the cervical or vulvar tissues that contained the DNA viruses showed the classic cytologic changes diagnostic of the diseases. Representative examples are provided in Fig. 1. Note the variable cell density, non-uniform perinuclear halos, and variability in nuclear size, shape, and chromaticity diagnostic of HPV-induced CIN 1. Also, the multinucleated squamous cells towards the surface that show the so-called ground glass nuclei are evident in the herpes simplex cases. Finally, the areas of inverted acanthosis with the large eosinophilic inclusions that are unique to the poxvirus molloscum contagiosum are readily apparent.

Productive infection for both HPV and herpes simplex virus was