EVs in HPV Infection

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Since their description, extracellular vesicles (EVs) have shown growing relevance in cancer progression. These cell structures contain and transfer molecules such as nucleic acids (including DNA and RNA), proteins, and lipids. Despite the rising information about EVs' relationship with cancer, there is still scarce evidence about their content and function in cervical cancer. Interestingly, the composition and purposes of some cellular molecules and the expression of oncogenic proteins packaged in EVs seem modified in HPV-infected cells; and, although only the E6 oncogenic protein has been detected in exosomes from HPV-positive cells, both E6/E7 oncogenes mRNA has been identified in EVs; however, their role still needs to be clarified. Given that EVs internalizing into adjacent or distant cells could modify their cellular behavior or promote cancer-associated events like apoptosis, proliferation, migration, or angiogenesis in receptor cells, their comprehensive study will reveal EV-associated mechanisms in cervical cancer.

HPV exosomes extracellular vesicles exosomal content E6/E7 cancer

1. Extracellular Vesicles in Cervical Cancer and HPV Infection

Extracellular vesicles (EVs) have been considered essential for developing several kinds of cancer and other pathologies in the last years. To date, there are thousands of articles evidencing EVs´ involvement in cancer progression [1][2]. However, studying cancer in such a general way is both impossible and inaccurate, so this review focuses on cervical cancer, which is still considered a global public health problem [3].

In 2020, 341,831 women died from cervical cancer all around the world. Among all types of cancer, 3.4% correspond to deaths from cervical cancer worldwide [4]. Cervical cancer is associated with high-risk human papillomavirus (HR-HPV) infection in 99.7% of cases [5]. HR-HPVs are considered the main predisposing factor, but it is not sufficient by itself for cancer evolution [6]. Cancer progression depends, among other factors, on multiple heterotypic cell interactions forming the tumor environment [7]. Thus, in addition to HPV infection, the contribution of several additional factors has been analyzed [6]. Recently, some analyses have evaluated the role of EVs in cancer development since these EVs lead to cellular communication through transferring their content from a donor to a receptor cell and the consequent modification of cellular processes allowing tumor progression [8].

EVs' existence was suggested in 1946, but was only widely demonstrated in the last four decades. Since their description, EVs have shown increasing relevance in cancer progression [9][10][11]. According to the International Society of Extracellular Vesicles (ISEV) definition, EVs are particles naturally released by cells. These elements are

delimited by a lipid bilayer, do not contain a functional nucleus, and cannot replicate [12]. Considering their biogenesis, the main types of EVs include exosomes, microvesicles (MVs), and apoptotic bodies [8][11].

When cells incorporate extracellular milieu content through early endosomes by invagination of the cell plasma membrane, these early endosomes mature into late endosomes by fusion with other vesicles. Then, multivesicular bodies (MVBs) arise by internal budding of the endosomal membrane to develop intraluminal vesicles, the future exosomes. This process is mediated by the Endosomal Sorting Complex Required for Transport (ESCRT) or a ceramide-dependent mechanism. Finally, MVBs fuse with the cell membrane releasing the exosomes into the extracellular space [1][13]. MVs are generated from the direct cytoplasmic membrane budding through cytoskeleton reorganization in an intracellular calcium concentration-dependent process. Increased intracellular calcium changes plasma membrane phospholipids distribution and activates cytosolic proteins involved in cytoskeleton remodeling. Then, the cytoskeleton-bound membrane is disrupted, and contractile, and cleavage proteins such as calpain are recruited, allowing membrane blebs formation and microvesicles release [2].

EVs contain different molecules such as nucleic acids, proteins, lipids, and metabolites. Transferring of EVs' content promotes reprogramming of recipient cell functions [8][11]. EVs' interaction with target cells can be through different mechanisms such as membrane fusion, endocytosis, phagocytosis, or cell membrane molecules [8][13].

EVs are enriched in some molecules such as cytoskeletal proteins, class I and II major histocompatibility complex (MHC) proteins, adhesion proteins (tetraspanins, integrins), heat shock proteins, and membrane fusion proteins (Rab, annexins) [8]. Proteins packing and sorting in EVs is a regulated process, where some proteins are involved in their biogenesis and secretion of a particular kind of vesicles; hence, this can be used as a marker to identify the EVs type [14]. Regarding nucleic acids, these EVs contain high molecular weight double-stranded DNA, and in cancer patients, EVs are enriched with tumor DNA [8]. The proposed mechanism for DNA loading in EVs is through micronuclei, which, due to their instability, collapses and exposes their content to the cytoplasm, where DNA could be transported to the MVBs by interaction with CD63 tetraspanin and finally loaded into exosomes [15]. Additionally, EVs are also enriched with several non-coding RNAs, mainly miRNAs. It has been proposed that these miRNAs could be packaged by different mechanisms, such as the neutral sphingomyelinase-dependent (nSMase2) exosomal transfer, sequence motifs recognition in miRNAs by post-translationally modified ribonucleoproteins, and by a pathway dependent on the post-transcriptional modification of the 3′ end of miRNAs [16][17][18].

2. relationship between EVs and HPV-associated cervical cancer

Despite all the available information on EVs' involvement in cancer, there are currently less than 50 experimental papers about their content in cervical cancer or the relationship between EVs and HPV-associated cervical cancer [8][19][20][21]. This evidence reveals the EVs' content from cervical cancer samples and cell lines.

Like in other illnesses, it has been demonstrated that EVs could contain proteins and nucleic acids. Even not all viral proteins have been localized in EVs; it has been possible to identify the E6 oncoprotein in EVs of CaSki cells,

a cell line of cervical cancer. In addition, it has also been determined that EVs can contain genomic and viral DNA. The RNAs in EVs include those from the cellular origin and viral mRNAs coding for E6/E7 oncogenes. Other molecules such as purine metabolites, amino acids, fatty acids, saccharides, and several other metabolites have been identified in exosomes derived from different cancers, mainly through mass spectrometry coupled to a previous separation method. The exosomes analyzed include those derived from head and neck squamous cell cancer (HNSCC), which is also related to HPV infection. But up to date, there are no reports about these molecules in cervical cancer [22][23].

EVs'characterization in cervical cancer opens broader prospects for its treatment, prevention, and prognosis. The identification of molecules contained in EVs would allow their use as markers of infection, viral integration, or disease progression. However, given the complex mixture of circulating EVs, the discrimination of their origin is very complicated. Hence, using EVs or their cargo molecules as biomarkers is still far from being their primary application. Descriptively, cervical cancer-derived EVs have been revealed as influencing hallmarks events of cancer such as angiogenesis, migration, and invasion, and probably other cell processes not related to this malignancy yet. Until now, cervical cancer therapy focused on eliminating transformed cells, but its recurrence and persistence may lead to patients' death. Hence, to some extent, cervical cancer relapse could be due to alterations induced by EVs in the tumor milieu or far away places via blood transportation.

The comprehensive EVs´ characterization and awareness of EVs´ functions will allow their application to lead to new methods to eliminate altered cells. However, manipulating EVs content or EVs design by microparticles engineering loaded with appropriate molecules requires further EVs characterization, standardizing internalization methods, confirming receptor molecules of uptake, and evaluating cell effects and scope in various cell types.

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