4. Cervical cancer and apoptosis

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Abstract. Cervical cancer is a global health problem that affects women of all ages. Cancer is defined as a disease that shows the following seven features: insensitivity to growth-inhibitory signals, high proliferative capacity, production of autocrine factors that promote its proliferation, invasion, metastasis, evasion of apoptosis and, recently, chronic inflammatory processes. Data from epidemiological, clinical and experimental studies sustain the conclusion that the human papillomavirus (HPV) is the main causative agent of this cancer. This section of the book will discuss the effect of HPV on the evasion of the apoptotic event as predominant mechanism for the initiation and transformation towards a malignant phenotype.

Apoptosis

Apoptosis is a type of programmed cell death responsible for eliminating cells that have been damaged and the viability of which has thus been compromised. This cellular destruction process is essential in the normal development and homeostasis of multicellular organisms. Under normal conditions, apoptosis can be activated via two pathways: the intrinsic one (also known as the mitochondrial pathway) and the extrinsic one (known as...
the death receptor pathway). The intrinsic pathway may be activated by different agents such as ultraviolet radiation, antineoplastic agents, etc. These factors induce the loss of integrity of the outer mitochondrial membrane, allowing the translocation of mitochondrial proteins such as cytochrome-c, Smac/DIABLO, Omi/Htra2 and AIF-1 to the cytoplasm. Two groups of proteins orchestrate the apoptotic process: Bcl-2 family members and caspases.

The Bcl-2 family is involved in regulating mitochondrial permeabilization; the family is divided into two groups: pro-apoptotic proteins (Bax, Bak, Bid and Bok/Mtd) and anti-apoptotic proteins (Bcl-2, Bcl-\(x_L\), Bcl-w, Mcl-1 and A1/Bfl-1). In basal conditions, caspases exist as inactive zymogens that are activated when cut by some cysteine protease (which can be themselves). Caspases can be grouped into two groups, the initiators (caspase-8 and -9) and the effectors (caspase-3, -7); as implied by their name, the first initiate the apoptotic process, while the latter are responsible for the degradation of specific substrates that induce the apoptotic phenotype. The cytochrome-c in the cytoplasm induces the formation of the apoptosome through the recruitment of Apaf-1 and pro-caspase-9, inducing the self-activation of caspase-9. This initiator caspase induces the activation of effector caspases by its cysteine protease activity, which leads to the apoptotic phenotype.

The coupling of a death receptor with its specific ligand activates the extrinsic pathway. For example, the union of CD95 by its ligand FasL induces the recruitment of the adapter protein fas-associated protein with death domain (FADD), by interactions between the death domains of both proteins. Caspase-8 is then recruited to the receptor through FADD. This complex is called the death inducing signaling complex (DISC) and it induces the self-activation of caspase-8. This caspase, in turn, induces the activation of effector caspases by its cysteine protease activity. It has been described that the extrinsic pathway may also induce activation of the intrinsic pathway, as Bid, a pro-apoptotic member of the Bcl2 family, is proteolytically cut by caspase-8, and is translocated to the mitochondria, inducing the activation of the mitochondrial pathway.

**Apoptosis regulation**

Apoptosis is regulated by different mechanisms; one of the most important is carried out by the family of inhibitor of apoptosis proteins (IAPs). Structurally, the members of this family have one to three BIR domains, which interact with and inhibit the active forms of caspases. Some IAPs have RING domains that present ubiquitin -E3 ligase activity,
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contributing to the degradation of protein targets. The most important and studied protein of this family is XIAP, which has three BIR domains and one RING. XIAP is able to inhibit caspases -3, -7 and -9. The overexpression of IAPs has been observed in different cancers as a mechanism for evasion of apoptosis. It has been demonstrated in samples of cervical cancer patients that XIAP protein expression correlates with the recurrence of the disease [1]. The same study found that Survivin, another member of the IAP family, increases its expression in cells with a malignant phenotype [1]. These results are consistent with those described for different types of cancer, supporting the idea that the over-expression of IAPs is a mechanism to evade apoptosis [2]. Smac/DIABLO an interesting mitochondrial protein, is a negative regulator of IAPs that induces the release of caspases retained by IAPs and favors the apoptotic event. Different studies have demonstrated that this protein has a low expression in the samples from patients with different types of cancer [3-6]. Espinosa, M et al. determined that Smac/DIABLO is expressed de novo in a subset of samples of cervical cancer patients [7]. When the number of patient samples was increased, the presence of Smac/DIABLO was associated with a high percentage of recurrence in samples from squamous cervical carcinoma [8]. This result could be considered contradictory because Smac/DIABLO is a negative regulator of the activity of IAPs. A plausible explanation is that the increased expression of Smac/DIABLO may be due to a compensatory response to the possible increase of IAPs. However, this study did not evaluate the presence of some IAPs [8]. This motivated us to propose that both pro-apoptotic and anti-apoptotic proteins must be analyzed in the same patient samples to obtain a comprehensive view of the samples in question [9]. Furthermore, it has been reported that Smac/DIABLO regulates other cellular processes independently of its ability to regulate apoptosis [10].

**Etiological factors in cervical cancer**

Worldwide, cervical cancer is the second most commonly diagnosed cancer in women. It has been estimated that 80% of the annual deaths attributed to this cancer occur in developing countries [11]. Many studies have been carried out to understand the etiology of cervical cancer since it was considered as a public health problem. Data from epidemiological studies have indicated that the infection by human papillomavirus (HPV) is associated with cervical carcinogenesis. However, HPV infections are benign and are resolved within a time span of 1-2 years without the application of any treatment. Because of these facts, HPV is conceptualized as a necessary but not sufficient agent to induce cervical cancer. The
presence of co-factors is critical to the establishment of the disease. These co-factors have an impact on the natural history of the lesion, inducing increased viral persistence and, consequently, its progression. These co-factors can be split into three groups: (A) related to the host, (B) related to behavior and (C) related to the environment. (Table 1).

Among the co-factors related to the host, the suppression of the immune system plays an important role. This has been clearly observed in individuals in which, due to different circumstances, the immune system is altered, such as HIV infected individuals [12], individuals undergoing an immunosuppressive treatment prior to organ transplantation [13], or pregnant women [14]. In this regard, it is reported that patients diagnosed with cervical intraepithelial neoplasia that had a regression to a normal phenotype showed higher levels of CD4+ T cells, a reactivation of the immune system [15]. On the other hand, several studies have postulated another risk co-factor for cervical cancer associated with the host but with heritable characteristics. However, these studies conclude that cervical tumors are best explained based on a multifactorial mechanism, which involves a heritable component [16, 17].

As HPV infection is a sexually transmitted disease, the sexual behavior is intrinsically related to the natural history of the infection. Undoubtedly, having sexual intercourse with different sexual partners increases the risk of recurrent infections with different variants of HPV and of a benign lesion progressing to a malignant one [11]. In addition, it has been reported that there may be an increased risk of being infected with HPV the younger a person starts its sex life, although this claim is related to the greater likelihood of having more sexual partners during a lifetime [18]. It is worth noting that the risk increases when there are sexual contacts with persons at

Table 1. Co-factors involved in cervical cancer.

| Factors related to the host | • Suppressed immune system.  
|                           | • Genetic predisposition.  |
| Factors related to the conduct | • Multiple sexual partners.  
|                               | • Age at first intercourse  
|                               | • Parity  
|                               | • Risk behaviors (sexual encounter with persons at risk)  
|                               | • Oral Contraceptives  
|                               | • Steroidal hormones.  |
| Environmental Factors | • Smoking.  |
risk, such as prostitutes. Moreover, because there are response elements to steroid hormones in the virus genomes, it is probable that the rate of viral gene transcription is influenced when hormones are present. In the case of HPV infections, the presence of steroid hormones influences the development of a malignant phenotype [19, 20]. It has been observed that samples from patients with high levels of steroid hormones are correlated with greater prevalence of HPV [21]. On the other hand, a positive relationship between parity and HPV has been observed in cervical cancers. It has been determined that women who deliver more than four children have a risk twice as high as those that conceive only one or none at all [22]. Hormonal, traumatic and immunological mechanisms have been proposed to explain this association [23].

Of the environmental factors, smoking is well understood as a risk factor for various cancers [24, 25]. Cigarette smoking has been taken by some studies as an independent risk factor for cervical cancer [26, 27]. The biochemical mechanism involved in smoking is thought to be related to DNA damage and mutation induction.

**Infection with HPV and cervical cancer**

Several epidemiological and experimental studies have confirmed that of the possible etiologic agents that have been associated with cervical cancer, HPV is the main cause of this cancer. The genome of the human papillomavirus is circular and double stranded, containing between 6800 and 8000 base pairs. This genome is contains eight open reading frames divided into three groups, the encoding genes of early proteins (E1-E7), the encoding genes of late structural proteins (L1 and L2) and the non-coding region which contains structural elements that function in cis for the replication and transcription of the viral genome. The human papilloma virus has variants which are classified into two main groups, the high-risk ones (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) and the low-risk (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81). The infection with high-risk HPV induces lesions that progress to high-grade epithelial neoplasms and finally develop carcinomas, while the infection with low-risk variants produces only genital lesions and rarely progress to a malignant phenotype. However, the lesions induced by the two groups can progress to cancer if they are recurrent and the co-factors described above are present.

In the early stages of infection, when the viral genome is episomal, the presence of the E5 protein of the HPV virus stimulates cell growth by interacting with the receptors of the epidermal growth factor B, derived from platelets, and of the colony stimulating factor 1 [28]. In an *in vitro* model of
cervical cancer, apoptosis was inhibited by E5 by means of an ubiquitination-dependent proteosomal degradation of the pro-apoptotic protein Bax, preventing the activation of the mitochondrial pathway [29]. Furthermore, by inhibiting the DISC assembly, E5 was able to decrease, via the extrinsic pathway, the activation of caspases -8 and -3 [30]. It has been observed that in the events in which the viral genome integrates into the host genome, the coding sequence of the E5 gene is deleted, which allows to infer that this gene is not required in late stages of the carcinogenic process by HPV infection.

Another protein that is deleted when the viral genome is integrated is E2. This protein has remarkable qualities; it inhibits the transcription of the viral genes E6 and E7, which are indispensable in the late stages of HPV infection. This action is mediated by the transcriptional activation domain located in the amino-terminal, and by the DNA binding domain located at the carboxyl-terminus [31]. It has been observed that the presence of this protein activates apoptosis through mechanisms that are dependent [32, 33] and independent of p53 [34, 35]. There is a difference between the E2 proteins of the high and low risk HPVs. The low risk proteins are constrained to a nuclear distribution, while the high-risk ones are also cytoplasmic due to CRM1, a carrier protein that shuttles proteins from the nucleus to the cytoplasm. In the cytoplasm, E2 is capable of inducing the activation of caspase-8, activating apoptosis [34]. However, it has been described that E2 is a substrate for the active form of caspases, so there is a negative feedback [36]. The deletion of E2 during the integration event is essential to the carcinogenic process induced by HPV.

The viral protein E6 has been linked to the carcinogenic process. E6 interacts with the p53 protein, inducing its degradation [37], which in turn creates chromosome instability since p53 checkpoint activity is lost when the DNA is damaged. E6 protein stimulates the activity of telomerase, which is a ribonucleoprotein with polymerase activity that synthesizes telomeric sequence repeats and prevents telomere attrition. The protein is important but not sufficient for the immortalization of cells [38]. In addition, E6 increases cyclin-dependent kinase activity (CDK) [39], which could paradoxically be a permissive factor for apoptosis induction. Nevertheless, during apoptosis, E6 degrades Bak, a pro-apoptotic member of the Bcl-2 family [40, 41], thus inhibiting cell death. Additionally, E6 induces the degradation of FADD and of pro-caspase-8, inducing resistance to the activation of the extrinsic pathway by inhibiting the formation of DISC and, consequently, the activation of effector caspases [42]. However, malignancy does not depend on the expression of E6 alone, since the presence of E7 is also necessary.
E2F is a transcription factor required for the activation of genes involved in the S-phase of the cell cycle. This factor is negatively regulated by the retinoblastoma protein (RB), which is inhibited by phosphorylation. The oncoprotein E7 interacts and degrades the retinoblastoma protein (RB) [43, 44], releasing E2F from the repression exerted by RB, inducing cell proliferation [45]. The E7 protein is associated with the stimulation of cyclin A [46, 47] and cyclin E genes [39], key regulators of cell growth. However, it has been reported that E7 can also induce p53 [48] and p21 [49]-dependent apoptosis, although it has been reported that the presence of E6 and E7 of high-risk HPV induces the transcriptional activation of cIAP-2 (a member of the IAPs family). cIAP-2 up-regulation is needed to protect cells from TNF-α-induced cell death [50]. E7 is able to induce the activation of the NF-KB pathway through p52 and, in turn, this cascade up-regulates cIAP-2 protecting these cells from apoptosis by TNF-α [51]. Paradoxically, the apoptotic effect of E7 seems to be associated with the activation of the extrinsic pathway. Yamato, et. al determined that the presence of E7 promotes apoptosis by TNF-α or TRAIL [52]. Further studies are needed to resolve this discrepancy. Additionally, the actions of E6 and E7 have been described as complementary in the sense that the inhibitory cellular mechanisms induced by the presence of a viral oncoprotein are supported by the actions of the other oncoprotein and vice versa. For example, the presence of INK4a inhibits the activity of E6, but E7, due to its stimulatory activity on cyclins, eliminates this regulatory mechanism. On the other hand, E7 can induce p53-dependent apoptosis, which is inhibited by the effect of E6 on p53 and the members of the apoptotic pathway mentioned above. Finally, the contribution of the rest of the viral proteins of HPV to the apoptotic event has not been clearly elucidated.

**IAPs and cervical cancer**

The evasion of apoptosis by over-expression of the members of the IAPs family is a mechanism that has been described for various types of cancer. The same situation has been observed in cervical cancer (Table 2). The survivin protein is a member of the IAPs family. This protein is expressed during the G2/M phase of the cell cycle. The antiapoptotic effects of survivin are mediated through the inhibition of caspases -3 and -7 [53, 54]. Additionally, this protein interacts with the microtubules of the mitotic spindle, being required for the mitotic checkpoint as part of the chromosomal passenger complex. Interestingly, survivin has been detected in human fetal tissues [55, 56] and in different types of neoplasia, but it is
**Table 2.** Expression of various IAPs and cervical cancer. IH, Immunohistochemistry, CI, immunocytochemistry, CIN, cervical intraepithelial neoplasia.

<table>
<thead>
<tr>
<th>IAP</th>
<th>Detection method</th>
<th>IAP expression/malignant phenotype</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survivin</td>
<td>IH</td>
<td>Co-localization of HPV and survivin in patient samples.</td>
<td>[58]</td>
</tr>
<tr>
<td></td>
<td>IH</td>
<td>Expression of survivin correlates lesions larger than 4 cm, lymph vascular space invasion and lack of response to initial therapy in patients.</td>
<td>[59]</td>
</tr>
<tr>
<td></td>
<td>IH</td>
<td>Direct correlation between the increase in the degree of injury and was associated with the presence of high risk HPV.</td>
<td>[60]</td>
</tr>
<tr>
<td></td>
<td>IH</td>
<td>Increased expression of survivin in cervical cancer with an inverse relationship between the expression of caspase-3.</td>
<td>[61]</td>
</tr>
<tr>
<td></td>
<td>IH</td>
<td>Survivin was detected in all cases with cancer. Patients with higher expression of survivin had an increase of 3.3 times die from cancer than those who had low levels. Study in samples from patients who received radiotherapy</td>
<td>[62]</td>
</tr>
<tr>
<td></td>
<td>IH</td>
<td>The expression of survivin was detected in all samples from patients treated with radiotherapy. Expression cytoplasm and nucleus was detected.</td>
<td>[63]</td>
</tr>
<tr>
<td></td>
<td>IH</td>
<td>The expression of survivin was overexpressed in cervical cancer samples. Correlated with levels of BCL2.</td>
<td>[64]</td>
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<tr>
<td></td>
<td>IH</td>
<td>Increased survivin expression in advanced stages of the disease. The expression of survivin and telomerase were associated with the severity of the lesion</td>
<td>[65]</td>
</tr>
<tr>
<td></td>
<td>IH</td>
<td>Overexpression of survivin in CIN and cervical cancer squamous type.</td>
<td>[66]</td>
</tr>
<tr>
<td></td>
<td>IH</td>
<td>Increased expression of survivin was detected in cervical cancer than in cervicitis and CIN groups. Inverse relationship between the expression of survivin and caspase-3</td>
<td>[67]</td>
</tr>
<tr>
<td></td>
<td>IH</td>
<td>Overexpression of survivin was detected in more samples with increasing degree of malignancy. Survivin expression was directly and inversely proportional to the expression of Fas and FasL.</td>
<td>[68]</td>
</tr>
</tbody>
</table>
barely expressed in the differentiated tissues. Two isoforms generated by alternative splicing of the messenger RNA of the survivin gene have been described, survivin-2B and Survivin-Dex3. The first retains 69 base pairs (bp) of intron 2, functioning as a cryptic exon, while the second loses exon 3, which has 118 bp. The expression of survivin-DEx3 has anti-apoptotic
potential, while Survivin-2B shows a reduction of the anti-apoptotic potential [57]. The expression of survivin is present in pre-cancerous and cancerous lesions, and is absent in normal tissue. This protein has been associated with a poor prognosis for patients with cervical cancer. XIAP is another interesting protein, the most important member of the IAPs. However, it has not been extensively studied in cervical cancer. Like other IAP family members, it has been found that XIAP confers resistance to various apoptosis-inducing agents such as antineoplasics, radiotherapy, and immunotherapy. The expression in cervical cancer of other IAP family members such as Livin, c-IAP1 and c-IAP2 has been analyzed (Table 2). Of the studies cited in this section, it is important to point out the one by the group of Indarti, J. et al., which analyzes the contribution of the expression of survivin as an additive risk factor for cervical cancer. With this integrative vision, they define a profile of those individuals who may be at risk of progressing to a more severe lesion. It is clear that this profile depends on the intrinsic characteristics of each population but is an example of the integration of various elements that have been presented in this chapter.

Caspases and cervical cancer

One of the main cancer hallmarks is the disruption of the critical balance of apoptosis and proliferation during carcinogenesis. As a consequence, damaged cells are not eliminated, predisposing the survival of these mutated cells and allowing them to participate in different selection processes that are necessary for the full tumorigenic phenotype. Initiator caspases are involved in triggering the intrinsic and extrinsic pathways of the apoptotic pathway. The importance of effector caspases is evident, since they degrade specific substrates which are proteins involved in basic cellular processes. Therefore, alterations in the expression of these caspases have an important role in the carcinogenic process. There are some studies that focus on the expression of caspases in cervical cancer. (Table 3). Interestingly, down-regulation of caspase-3 and caspase-9 seems to be clearly associated with worse clinical status. Contradictory, some studies had demonstrated a direct relationship between caspase detection and cervical cancer (including pre-cancerous lesions). It would be interesting to determinate if there were any change in IAPs protein levels that could counteract this increment.
Table 3. The relationship between caspases and cervical cancer. CIN, cervical intraepithelial neoplasia.

<table>
<thead>
<tr>
<th>Caspase-</th>
<th>Direct relationship between status disease and caspase (expression or activity).</th>
<th>Inverse relationship between status disease and caspase (expression or activity).</th>
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<tbody>
<tr>
<td>3</td>
<td>• Increases the severity of CIN [76]. • Increased expression in cervical cancer than in the group of samples with cervicitis or CIN [67]</td>
<td>• Undetectable in samples of adenocarcinoma and adenosquamous carcinoma type [77]. • Low expression with increasing pathological grade and clinical status [79]. • Decreased expression of high-grade CIN lesion to cervical carcinoma [76]. • Decreases active caspase-3 expression in dysplasia and invasive cervical carcinoma [79]. • Low expression in high-grade disease in advanced clinical status and lymph node metastasis [74].</td>
</tr>
<tr>
<td>9</td>
<td>• Higher activity enzyme in low-grade CIN lesions than in controls [80].</td>
<td>• Undetectable in samples of adenocarcinoma and adenosquamous carcinoma type [77]. • Decreased enzyme activity in CIN and high-grade lesions greater decrease in cervical cancer [80].</td>
</tr>
<tr>
<td>6</td>
<td>• Enzyme activity increased in low-grade CIN [81].</td>
<td>• Low expression with increasing pathological grade yestado clinical [78]. • Decreased expression of high-grade CIN lesion to cervical carcinoma [76].</td>
</tr>
<tr>
<td>2 and 5</td>
<td>• Increased enzyme activity in low-grade CIN lesions than in controls [80].</td>
<td>• Inverse relationship between the expression of caspases and cervical cancer [80].</td>
</tr>
</tbody>
</table>

**Reactivation of apoptosis as cancer therapy**

Modulating the apoptotic event in cervical tumor cells is an option for controlling this disease. The development of small interfering RNAs (siRNAs) targeted to oncoviral proteins of HPV is a viable tool for preventing their evasion of apoptosis. This assertion has been successfully observed in *in vitro* models for E6 [82-85] and E7 [86, 87] activity. In addition, restoring the p53 pathway by nonsteroidal anti-inflammatory molecules [88] or by proteasome inhibitors [89, 90] has been proposed. Interestingly, immunotherapy gives us the opportunity to have prophylactic and therapeutic effects simultaneously. The development of vaccines of viral chimeric particles between the antigenic epitopes of E7 and L1 has met with success in cervical cancer mouse models by generation of both humoral
response and cell-mediated immunity [91]. Another approach is to fuse the DNA binding domain of the oncoprotein E2 to the catalytic domain of the endonuclease Folk. Endonuclease enzyme activity was detected in HeLa cells in the E2 binding sites in the integrated viral DNA of HVP18 [92]. Since effective treatment options are limited in advanced cervical cancer, the discovery of new targets and the development of new small molecules are needed for clinical testing and, finally, cancer cervical treatment.

**Conclusion**

It is well known the association between HPV and cervical carcinogenesis. In principle, HPV infections and premalignant lesions could be resolved without any treatment, but the presence of risk factors, as described above, allows the transition from pre-cancerous lesions to malignant tumor. HPV oncoproteins alter cellular homeostasis by diverse molecular mechanism that modify the apoptotic response. HPV directly or indirectly modulates protein levels of IAPs and/or caspases, prompting a cellular resistance to apoptosis in cervical carcinogenesis. HPV vaccines with prophylactic purposes are available to reduce disease incidence, however, when the disease is established, current therapeutic treatments are still ineffective. Understanding how HPV modulates apoptosis during disease progression will open the door to improve the designing of small molecules to target the disease.

**References**


