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Summary and General Discussion
Immune surveillance is of utmost importance in preventing cervical carcinogenesis. Cytokines play a central role in directing and fine tuning the immune response. In cancer, cytokines can either be involved in stimulating the anti-tumor immune response or in tumor growth and progression. Certain cytokines play a dual role. For example, TGF-β acts as a tumor suppressor early in carcinogenesis and as a tumor promoter late in carcinogenesis. The studies presented in this thesis concern molecular analyses of immune escape mechanisms besides HLA class I loss, such as loss of TNFα expression and TGF-β insensitivity in cervical cancer. Furthermore, we studied genome-wide molecular alterations to identify possible genes involved in cervical carcinogenesis, including genes involved in the immune response. In this chapter, the results obtained are summarized and put into perspective.

The primary cause for the development of cervical cancer is infection with human papillomavirus (HPV). However, the majority of HPV infections and cervical intraepithelial neoplasia (CIN) are cleared by cellular mediated immune responses [1, 2]. A deterioration of the immune status of a person likely predisposes to persistence of infection, the formation of CIN lesions and a higher risk for the development of cancer [3]. A well-known strategy of neoplastic cells to evade cellular mediated immunity is loss of antigen presentation via human leukocyte antigens (HLA) class I [4]. Nevertheless, some cervical tumors escape immune surveillance without loss of HLA class I [5].

To investigate the immune escape mechanisms utilized by HLA positive tumors, in Chapter 2 the gene expression profile of cervical tumors with no/minimal loss, partial loss and total loss of HLA class I, measured by microarray, is described. A significant difference in expression of 150 genes was shown between tumors with total loss and no/partial loss of HLA class I. The Gene Ontology (GO) biological process that encompasses most of these genes is defense response. HLA positive tumors showed a significant increase of genes involved in proinflammatory and acute phase response. When a difference in the extent of infiltrate between the 2 groups (HLA negative and HLA positive tumors) was included as a possible confounder in the analysis, defense response, including proinflammatory and acute phase response, remained significantly higher in HLA positive tumors. Besides genes involved in an inflammatory response also a number of interferon γ (IFNγ) induced genes were upregulated in HLA positive tumors. A possible reason for this phenomenon would be that immune cells attracted by the tumor become activated by the presentation of viral and/or tumor-derived peptides and start producing IFNγ. IFNγ may also activate tumor associated macrophages (TAMs) which in turn start producing proinflammatory cytokines and chemokines [6, 7]. In this scenario the question remains how cells from the immune system in HLA positive tumors become activated. We speculate that in contrast to HLA negative tumors, activated cytotoxic T lymphocytes present in the tumor destroy HLA positive tumors, resulting in debris. This debris is taken up by antigen presenting cells (APC), resulting in activation of the APC. Then, these APC present (viral) antigens to T helper lymphocytes which in turn become activated and start producing cytokines and interferons. Although this sequence of reactions would sustain an anti-tumor response [8], apparently this immune response is not sufficient
to eradicate the tumor. A recent paper by de Boer et al. showed a strong positive correlation between HLA class I expression and HPV-specific immune response [9]. This observation emphasizes the importance of HLA class I expression in maintaining anti-tumor immunity.

Although proinflammatory cytokines and chemokines play an important role in attracting and stimulating immune cells, these molecules may also contribute to tumor development by functioning as tumor growth factors or by stimulating angiogenesis [10,11]. In cervical cancer, expression of the CCL2 (also known as MCP-1), a chemoattractant of monocytes, associated with the presence of TAMs and a decreased overall survival [12], suggesting a role for this chemokine in tumor progression.

Since cytokines are pleiotropic and work in concert, it is difficult to predict whether the proinflammatory environment will benefit the tumor or will sustain an anti-tumor immune response [13,14]. This will also be dependent on tumor type and stage. According to our results, genes associated with defense response that differed significantly in expression between HLA positive and HLA negative tumors, encoded a number of chemokines and 2 acute phase proteins. Especially the serine protease inhibitors SerpinA1 and SerpinA3 showed a major difference in gene expression. High SerpinA1 and SerpinA3 expression in HLA positive tumors was confirmed by real-time PCR and immunohistochemistry. Other serine protease inhibitors such as PAI-1, maspin and headpin can exert tumor inhibiting or tumor promoting activity [15-17]. In cervical cancer, PAI-1 expression in the tumor was shown to be a prognostic unfavorable factor [18]. This may be due to its stabilizing effect on matrix composition which may facilitate the tumor by providing growth factors and supporting angiogenesis [17,19]. In inflammatory processes, SerpinA1 and SerpinA3 protein levels are often increased in plasma [20]. Studies have shown that SerpinA1 acts as a potent anti-inflammatory agent by inhibiting LPS-induced macrophage activation in vitro and suppressing nuclear transcription factor-Kappa B translocation [21-23].

We observed that protein expression of either SerpinA1 or SerpinA3 in HLA positive tumors correlated with poor prognosis. Previously, high protein expression of SerpinA1 in colorectal and lung adenocarcinomas was shown to be a poor prognostic factor [24,25]. SerpinA3 protein expression has been detected in gastric and hepatocellular carcinoma [26,27]. Possible mechanisms by which SerpinA1 and SerpinA3 could be involved in tumorigenesis are still poorly understood. It has been suggested that SerpinA1 plays a role in immune suppression since it caused strong inhibition of NK cytotoxicity in vitro [28]. In accordance with this observation, SerpinA1 was shown to inhibit granzyme B, a trypsin-like protease [29]. Thereby, SerpinA1 could restrain anti-tumor immunity through inhibition of the cytotoxic potential of immune cells. In the case of SerpinA3, inhibition of apoptosis is a suggested mechanism by which tumorigenesis could be promoted. In hepatoma cells, SerpinA3 inhibited apoptosis by inactivating chymotrypsin [30]. Since HLA positive tumors might be targets for cytotoxic T lymphocytes, expression of the SerpinA1 and/or SerpinA3 by the tumor cells may provide the tumor with an advantage by promoting survival and/or immune suppression. However, when not taking HLA status into account, SerpinA1 or SerpinA3 did not significantly affect survival or clinical parameters. Since SerpinA1 and SerpinA3

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participate in a large network of cytokines, chemokines and other immune regulatory mediators, it is conceivable to assume that these Serpins have a limited influence on the final outcome of a tumor promoting or tumor inhibiting environment.

**Tumor necrosis factor α**

Tumor necrosis factor α (TNFα), a cytokine with strong proinflammatory properties was demonstrated to be expressed in normal cervical epithelium, but not in most CIN and only in about 50% of cervical tumors [31]. The TNFα gene is located at chromosome region 6p which is affected by LOH in about 50% of cervical tumors [5]. Based on these findings, we hypothesized that TNFα could be a classical tumor suppressor gene. To investigate whether loss of TNFα, as a mechanism to escape immune clearance, is due to (epi)genetic inactivation of TNFα, loss of TNFα expression was studied in association with (epi)genetic characteristics in Chapter 3. TNFα mRNA expression, as studied by RISH, was found in 55% of tumors, which is in accordance with previous data [31]. However, no significant association was observed between loss of TNFα expression and genetic inactivation, as studied by microsatellite analysis of the respective locus and mutation analysis. Epigenetic inactivation by means of CpG promoter methylation did not reveal a correlation with lack of TNFα expression. Whereas our study shows that genetic inactivation as a cause for loss of TNFα expression is unlikely, we cannot exclude that promoter and exon 1 CpG demethylation is a prerequisite for transcription but insufficient on its own. Dependence on several epigenetic factors such as histone acetylation, histone methylation, and DNA demethylation, either acquired during cell differentiation and/or stimulation [32] and/or presence of tissue and stimulus-specific transcription factors may determine the outcome of TNFα mRNA expression in cervical epithelium [33, 34]. Furthermore, we investigated whether the TNF2 allele, which has been associated with upregulated TNFα expression, was preferentially targeted by LOH. Our data did not reveal preferential loss of the TNF2 allele, nor did we observe an association with high TNFα expression in cases carrying the TNF2 allele. Eight polymorphisms have been described within the TNFα promoter which could affect the binding of transcription factors, influence the activity of the promoter and resulting RNA and protein levels [35]. The functional significance of these polymorphisms remains to be elucidated. As for the TNF2 allele, characterized by the -308 G/A TNFα gene promoter polymorphism, conflicting results were obtained with studies associating this polymorphism with disease susceptibility [35]. In cervical cancer, 3 out of 4 studies did not observe an association of the TNF2 allele with cervical cancer, which is in accordance with our data [36-38]. Thus TNF2 does not seem to play a role in cervical cancer.

Besides regulation of TNFα expression, the question remains what role TNFα plays in cervical cancer. Nowadays, development and progression of a number of cancers is thought to be driven largely by chronic inflammation [39] of which TNFα is an important mediator [40,41]. However, in contrast to most tumors, the primary cause for development of cervical cancer is persistent infection with high risk HPV. HPVs are well able to subvert the immune response [42,43]. There is evidence that an inflammatory environment is suppressed and an immunosuppressive environment is
stimulated by relative downregulation of TNFα and upregulation of IL-10 in CIN compared to normal cervix [44]. Thus TNFα, which may interfere with HPV gene expression [45] and stimulate an immune response against HPV, could restrain persistence of the virus and thereby the development of cervical cancer. Once the tumor is established however, TNFα could function as a tumor promoting factor. For example, TNFα promotes tumor remodeling by stimulating fibroblast and macrophage activity, tumor cell motility and invasion via the induction of matrix metalloproteinases [46-48]. In cervical cancer, Zijlmans et al. showed a positive association between TNFα expression and stromal TAMs [31]. These TAMs at the tumor site are thought to produce various cytokines and chemokines [49] that could promote tumor progression. TNFα mRNA expression in cervical cancer however was not a prognostic factor, as observed by us and Zijlmans et al. Further investigation is needed to determine whether TNFα anti-tumor or pro-tumor characteristics prevail in cervical cancer.

Transforming Growth Factor β

Transforming growth factor β (TGF-β) is a cytokine with immune and growth inhibitory properties [50,51]. Early in carcinogenesis TGF-β functions as a tumor suppressor by inhibiting cell growth whereas late in carcinogenesis TGF-β is thought to function as a tumor promoter by stimulating invasion and metastasis [52,53]. TGF-β is expressed by various tumor types including cervical cancer [54-56]. During cervical carcinogenesis, evidence exists that overexpression of TGF-β is accompanied with decreased sensitivity for the growth-limiting effect of TGF-β [57-59]. Alterations in TGF-β signaling can be caused by inactivating mutations in TGF-β signaling components. In particular inactivation of TβR-II is frequently observed in colon cancer with microsatellite instability (MSI) and inactivation of the Smad4 tumor suppressor gene in pancreatic and non MSI colon cancers [60-62]. In cervical cancer, inactivating mutations of TGF-β receptor and Smad4 have been reported mainly in cell lines at low incidence [63,64]. Alterations in TGF-β signal transduction may also be due to HPV E7 oncoproteins that bind to Smad proteins (e.g Smad2, Smad3 and Smad4) and subsequently interfere with Smad transcriptional activity by blocking binding of Smad3 protein to its DNA target sequence [65,66]. In addition, E7 may interfere with TGF-β signal transduction by blocking TGF-β suppression of c-myc transcription via pRb [67]. Inhibition of TGF-β induced suppression of c-myc transcription and inhibition of Smad DNA binding activity by E7 oncoproteins was associated with resistance to the anti proliferative effect of TGF-β [66,67]. Other mechanisms that could influence TGF-β insensitivity are defects in the TGF-β pathway downstream of Smad signaling and crosstalk of TGF-β-Smad signaling with other pathways [68,69]. TGF-β signaling can activate MAPK signaling directly, independently of Smad, and pathways like MAPK, PI3K and Wnt can influence the outcome of TGF-β signaling by crosstalk [69-71]. Many of these pathways are involved in the regulation of cell growth and cell death. This interplay of TGF-β with other pathways could enhance carcinogenesis. For example, the invasive growth of Ras-transformed epithelial cells in vitro depends on intact autocrine TGF-β signaling [72].

To gain insight in the resistance to TGF-β induced cell growth inhibition in cervical
cancer, we determined the effect of TGF-β on the genome-wide mRNA expression profile of cervical cancer cell lines with different sensitivity to TGF-β in Chapter 4. A large-scale gene expression profile was determined using cDNA microarrays of cell lines with low, intermediate and high sensitivity to TGF-β-induced growth inhibition after 0, 6, 12 and 24 hours of stimulation with TGF-β. TGF-β-altered pathways involved in cell growth regulation that affected all cell lines included Smad, MAPK, TNFα and Wnt. In all cell lines, genes that negatively regulate cell growth were induced of which many are downstream targets of Smad signaling. Only in HeLa, the cell line with high resistance to TGF-β sensitivity, genes that positively influence cell cycle progression, namely *cyclin B2* and G2, were upregulated. Also evidence for TGF-β-induced signaling independent of Smad was present. MEFC2, a target gene of p38 MAPK, was upregulated [73]. This could be mediated by TGF-β induced activation of TAK1 which is a potent activator of SAPK/JNK and p38 MAPK [74-76]. Overall, HeLa showed most of the changes in expression of genes involved in MAPK signaling. This is in line with data from Malieka et al., who showed that reduced sensitivity of HeLa to TGF-β-induced cell growth inhibition is due to activation of MAPK [77]. In addition, TGF-β can interfere with TNFα signaling or promote TNFα-induced apoptosis [78,79]. In HeLa and SiHa, cell lines with low and intermediate sensitivity to TGF-β respectively, downregulation of genes coding for receptor, adapter and signaling molecules of the TNFα pathway, partially involved in apoptosis, was observed. Downregulation of signaling components in the TNFα pathway, involved in death signaling, may interfere with TNFα induced apoptosis and possibly play a role in TGF-β resistance. Furthermore, increased gene expression in receptor, signaling and target molecules of the Wnt pathway, a pathway associated with cell growth and development, was observed especially in HeLa. This may indicate TGF-β-induced activation of this pathway. Since the pathways discussed above are involved in cell growth and cell death and have crosstalk with TGF-β signaling, they could play a role in reduced TGF-β-induced growth inhibition.

Subsequently, we investigated an association between decreased sensitivity to TGF-β-mediated cell growth inhibition and TGF-β production in the cell lines. Only the cell lines with intermediate and low sensitivity to TGF-β produced TGF-β. The cell line most resistant to TGF-β, HeLa, showed the highest concentration of TGF-β in the supernatant. Addition of anti-TGF-β did not change cell growth kinetics. Thus cell lines which are refractory to TGF-β-induced growth inhibition do secrete TGF-β in a latent form which does not affect cell growth but probably contributes to paracrine stimulation of tumor development.

TGF-β canonical pathway

In cervical cancer, some defects in the TGF-β-Smad signaling pathway have been reported [63,64] but the exact role of TGF-β-Smad signaling remains unclear. In Chapter 5 we investigated protein expression of Smad2, Smad2P, Smad4 and p21, a major target of TGF-β-Smad signaling [80], in a large group of cervical carcinomas. All cervical carcinomas, assembled in a tissue array (n = 118) expressed Smad2, Smad2P and Smad4, as determined by immunohistochemistry. Also nuclear staining
of p21 was observed in the majority of tumors ($n = 99$). These data suggest a functional TGF-β-Smad signaling pathway in most cervical carcinomas. Nonetheless, cytoplasmic staining was weak for Smad2 in 33% and for Smad4 in 36% of tumors. To establish the basis for the weak Smad2 and Smad4 protein expression, we have investigated whether this was due to haplo-insufficiency of the Smad genes caused by loss of heterozygosity (LOH) or inactivating mutations. From the 46 cases analyzed, 40% showed loss at the locus of Smad2 and Smad4 which did not associate with weak Smad2 or Smad4 protein expression. Furthermore, no mutations in the functional MH1 and MH2 domains of Smad2 or Smad4 were observed in tumors with weak expression and/or absent nuclear staining.

In cancer, most mutations of Smad2 and Smad4 occur in the MH1 and MH2 domain, thereby affecting phosphorylation, nuclear translocation or protein stability [81,82]. One other study investigated Smad2 and Smad4 mutations in cervical carcinomas and reported one genetic aberration; an insertion in the MH2 domain of Smad2 in 1 tumor, resulting in loss of expression [64]. No genetic alterations were found of Smad4 in 20 tumors investigated [64]. These and our data suggest that genetic mechanisms for downregulation of Smad2 and Smad4 expression in cervical carcinomas are uncommon.

An epigenetic mechanism for downregulation of expression, methylation of CpG islands in the promoter region of Smad4 was studied in cervical cancer cell lines, normal samples and tumor samples. None of the CpG sites investigated were methylated. A few studies investigated CpG methylation of the Smad4 promoter as a mechanism for downregulation of expression in cancer [83-86]. These studies investigated different but overlapping areas of the promoter and the data obtained was not conclusive. Perhaps other epigenetic mechanisms, possibly in addition to methylation, such as histone acetylation and other chromatin remodeling machinery, may be required.

Furthermore, we investigated whether the expression pattern of Smad2, Smad2P and Smad4 associated with clinico-pathological parameters and survival. Smad4 weak cytoplasmic staining and lack of Smad4 nuclear staining correlated with tumor size and poor survival. In other tumors such as pancreatic and colorectal cancer, absent Smad4 protein expression was shown to be an unfavorable prognostic factor [87,88]. Low presence of Smad4 in the nucleus (and cytoplasm) of cervical tumors could mean that Smad4 target genes are insufficiently induced upon TGF-β signaling. Some of these target genes, such as p15 and p21, are involved in cell growth inhibition [51]. Surprisingly, Baldus et al. reported that all cervical cancer cell lines analyzed showed either absent or moderate responsiveness to TGF-β irrespective of their Smad4 status [63]. This has also been reported for colorectal and pancreatic cancer cell lines [89,90]. In addition to growth regulation, other tumor suppressing properties have been attributed to Smad4. Schwarte-Waldhoff et al. showed that the tumor-suppressive role of Smad4, in vivo, associated with morphological changes and improved adhesive properties of tumor cells [89]. This was accompanied with a reduced expression level of uPA and PAI-1, proteins that are implicated in the control of cell adhesion and invasion [19]. Smad4 was also shown to inhibit angiogenesis in vivo by reducing the expression of an important angiogenesis inducer, vascular endothelial
growth factor, and inducing the angiogenesis inhibitor thrombospondin 1 [90]. Thus besides being involved in growth inhibition, Smad4 exhibits other tumor suppressive properties which may be more important in cancer because often tumor cells acquire resistance to growth inhibition due to constantly active proliferating pathways like Erk MAPK [91].

Overall, Chapter 4 and 5 provide evidence that the canonical TGF-β-Smad pathway in cervical cancer does not seem to harbor major deficits (e.g. mutations in Smad2 and Smad4) but suggests that one of the contributory causes for alterations in signal transduction is crosstalk with other pathways and/or direct TGF-β-induced stimulation of these pathways, thereby altering/overruling signaling via Smads.

**Genome-wide molecular alterations in cervical cancer**

Host genomic alterations in addition to HPV infection are needed for precursor lesions to progress to invasive cancer. The accumulation of these genomic alterations is enhanced by the increased genomic instability that occurs as a result of high E6 and E7 expression after viral integration [92]. These include aberrations that affect tumor suppressor genes or oncogenes critical for tumor development. Multiple genetic alterations have been found in cervical cancer mainly by methods like metaphase comparative genomic hybridization (mCGH) and microsatellite marker analysis for the detection of LOH [93]. These techniques have resulted in the discovery of only a few genes, targeted by genomic imbalances and possibly involved in carcinogenesis, due to the lack of resolution and laborious work involved. Nowadays, techniques that allow detailed genome-wide analyses have become available, including array comparative genomic hybridization (array CGH), single nucleotide polymorphism array (SNP array) and gene expression arrays.

In Chapter 6, the genomes of 10 cervical cancer cell lines were examined in order to evaluate common regions of gains and losses and to identify genes involved in cervical carcinogenesis, particularly in immune response. Complex large-scale genetic changes were observed in the cancer cell lines. Many of these genetic alterations such as gain of 5p and 20q and loss of 4p and 13q, have been reported frequently in cervical cancer [94-96]. Most chromosomes were affected by LOH. In the cell lines, chromosome arm 6p belonged to those regions targeted most frequently by LOH, which has been reported previously [97]. A number of genes involved in immune regulation and antigen presentation are located at 6p including TNFα, HLA and peptide transporter TAP. The tumor probably benefits from loss of 6p since it may facilitate evasion of anti-tumor immune responses.

Genome-wide, no correlation between copy-number changes and mRNA expression were observed, suggesting that other mechanisms for transcriptional regulation are probably also important. Amplification of 5p though, correlated with a significant higher expression for 22% of the genes. Genes at 5p, such as SKP2, TRIO and ANKH, have been previously reported in association with other cancers [98-100]. Besides amplifications of these genes in different types of neoplasms, SKP2 and TRIO were shown to promote tumor cell proliferation [100,101]. Recently it has been shown that SKP2 is involved in TGF-β signal transduction. Upon TGF-β
stimulation, SKP2 is rapidly degraded, through signaling by the canonical Smad pathway, which results in stabilization of the cell cycle arrest protein p27, thereby ensuring TGF-β-mediated cell cycle arrest [102]. However, in a recent paper that investigated p27 and SKP2 protein expression in a large series of cervical carcinomas (n = 332) no correlation between p27 and SKP2 expression was found. Tumor stage was the only parameter with prognostic significance in case of SKP2 expression [103]. Future research has to reveal whether this oncogene is involved in cervical carcinogenesis for instance by investigating its effect on growth inhibition upon siRNA mediated knock down of SKP2 in cervical cancer cells.

Conclusions

The studies described in this thesis focus on molecular aspects of immune escape mechanisms in addition to HLA class I loss, including loss of TNFα expression and TGF-β insensitivity in cervical cancer (Figure 1). Tumors with partial and normal HLA expression as compared to tumors negative for HLA class I showed an overall higher expression profile of genes involved in proinflammatory responses such as the chemokines CCL3 and CCL4 and acute phase proteins SerpinA1 and SerpinA3. We identified SerpinA1 and SerpinA3 as candidate genes involved in immune suppression and/or carcinogenesis in HLA positive tumors. Besides HLA class I loss, we observed that loss of TNFα expression is a frequent event in cervical cancer. Based on our findings we can conclude that genetic inactivation of TNFα as a cause for lack of expression of TNFα is unlikely. The epigenetic regulation of TNFα expression and the functional significance in cervical cancer remain to be elucidated. Using an in vitro model, we have shown that TGF-β insensitivity in cervical cancer cells is associated with activation of pathways involved in proliferation like MAPK. Therefore, we speculate that TGF-β insensitivity in cervical cancer is caused by changes in signal transduction due to crosstalk of the TGF-β-Smad pathways with these pathways and/or TGF-β-induced stimulation of these pathways. In accordance with this view, we provide evidence by means of an immunohistochemical study of Smad protein expression that the TGF-β-Smad signaling pathway is functional in most cervical carcinomas and that inactivation of Smad2 or Smad4 genes as a cause for TGF-β insensitivity is unlikely. Finally, we aimed at identifying genome-wide alterations and genes involved in cervical carcinogenesis. A genome-wide analysis of cervical cancer cell lines on the DNA and RNA level indicated 3 candidate genes, SKP2, TRIO and ANKH at 5p that might be involved in the pathogenesis of cervical cancer. In conclusion, our studies provide evidence that cervical cancer cells develop various strategies to evade eradication by immune cells. These include loss of HLA class I, lack of TNFα expression, resistance to TGF-β growth inhibition, production of TGF-β and possibly SerpinA1 and/or SerpinA3 expression. Therefore, when treating cervical cancer patients with immunotherapy, one should take in account the array of escape tactics that cervical cancer cells employ.
Figure 1. Investigated immune escape mechanisms. 1. Cervical tumors show frequent loss of HLA class I expression. Immune escape routes in HLA class I positive tumors were investigated. HLA class I positive tumors showed strong expression of SerpinA1/SerpinA3 as a possible strategy of the tumor to escape immune surveillance (chapter 2). A common genetic alteration resulting in loss of HLA class I is loss of heterozygosity (LOH) at 6p21.3, the locus of HLA class I and TNFα. 2. Loss of TNFα expression due to LOH, mutation and/or promoter methylation was investigated. Genetic inactivation as a cause for lack of TNFα expression is unlikely (chapter 3). 3. Loss of TGF-β sensitivity. Resistance to TGF-β-induced growth inhibition is accompanied with stimulation of growth promoting pathways (MAPK and WNT) that could possibly counteract or overrule the growth inhibiting signal of TGF-β via Smads (chapter 4). Low cytoplasmic and absent nuclear staining of Smad4, a member of the canonical TGF-β pathway, may influence cervical carcinogenesis since it correlated with poor outcome (chapter 5), emphasizing the importance of the integrity of the canonical TGF-β pathway. 4. Performing genome-wide analysis (chapter 6), we observed amplification and overexpression of SKP2, a candidate oncogene which plays a role in TGF-β signaling since it needs to be degraded in order to accomplish TGF-β mediated cell cycle arrest.

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