Appendix: Colour Illustrations
Chapter 1, Figure 1. Cytokines involved in differentiation of helper T lymphocyte subsets. Abbreviations: DTH, delayed type hypersensitivity; Ab, antibodies. From Chabagolty et al., 2007.
Chapter 1, Figure 2. TGF-β induced activation of Smad and MAPK and their interactions. Activation of Smad signaling occurs once TGF-β binds to the TGF-β receptor type II (TβRII). Subsequently, TβRII phosphorylates TGF-β receptor type I (TβRI) which in turn phosphorylates Smad2/3. Then, activated Smad2/Smad3 bind Smad4 and translocate to the nucleus where the complexes function as transcription factors. Transcription is controlled by the presence of coactivators (Co-A) or corepressors (Co-R). Activation of the 3 MAPK (ERK, JNK and p38) by TGF-β or other stimuli such as growth factors or proinflammatory cytokines, can regulate Smad activation by direct phosphorylation or through downstream effector molecules. Examples of downstream effector molecules are cjun and ATF2 which modulate transcriptional activity. From Javelaud et al., 2005.

Chapter 1, Figure 3. TGF-β switches from tumor suppressor in normal and premalignant stages of tumorigenesis to proto-oncogene at later stages of disease. Progression to metastatic disease is usually accompanied by decreased or altered TGF-β responsiveness and increased expression/activation of TGF-β. Besides changes in the responsiveness to TGF-β in tumor cells, TGF-β effects tumor stroma in such a manner that tumor growth and metastasis is facilitated. From Roberts et al., 2003.
Chapter 2, Figure 4. Immunohistochemical analysis of SerpinA1 and SerpinA3 protein expression in cervical carcinomas. Arrows indicate tumor areas. A) Strong staining of SerpinA1, B) weak staining of SerpinA1, C) lack of SerpinA1 staining, D) strong staining of SerpinA3, E) mostly weak staining of SerpinA3 and F) lack of SerpinA3 staining.

Chapter 2, Figure 5. Immunofluorescent staining for SerpinA3 and HLA class B/C. Tumors are depicted with A) positive staining for HLA class I (red, membranous) and SerpinA3 (green, cytoplasmic) or B) no positive staining for HLA class I and SerpinA3, whereas infiltrate cells show positivity.
Chapter 3, Figure 1. TNFα mRNA expression in cervical cancer and normal cervical squamous epithelium (x 100). mRNA expression was measured by RISH as described in Materials and methods. The arrows point to tumor areas. A) Squamous cell carcinoma showing no TNFα expression, B) Squamous cell carcinoma showing low TNFα expression, C) Adenocarcinoma showing high TNFα expression and D) Normal cervical squamous epithelium showing TNFα expression.
Chapter 3, Figure 2. LOH at 6p21.3. A) Microsatellite analysis at 6p of 63 samples, as described in Materials and methods. B) The percentage of LOH for each marker. C) LOH profile of tumor 31. This tumor contained a diploid and aneuploid subpopulation. The first panel represents the normal (V+K-) subpopulation of the tumor that is heterozygous for the three markers: D6S265, TY2A and TNF. The arrows point to the different alleles. The black peak, visible in the graphs of TNF, is a size marker. The second panel, represents the diploid tumor (V-K+) subpopulation with an allelic imbalance (A.I.) of −2 at 6p, depicting an apparent decrease in one of the alleles for the 3 markers. The third panel shows complete loss of the allele for the three markers in the aneuploid tumor cells. The two graphs below illustrate the flow sorted subpopulations of the multiploid tumor. In green, the V+K- fraction, consisting of normal cells, is depicted (left graph). In red, the K+V- fraction, representing the tumor cells, is visible. As shown in the right graph, most of the tumor cells are aneuploid and a few are diploid, whereas the normal cells are all diploid.
Chapter 4, Figure 4B. TGF-β1 induced changes in gene expression of pathways in HeLa, SiHa and CC10B. Pathways, shown in light gray boxes, are associated with TGF-β1 signaling and involved in regulation of cell proliferation. The boxes below the pathways show in red, genes which are upregulated in expression, in blue, genes which are downregulated and in black, genes which show evident upregulation and downregulation during the 4 time points compared to time point 0 hr. In HeLa SSCG are observed in MAPK, Smad, Wnt, TNFα, NFKB and Rho pathways, in SiHa SSCG are observed in MAPK, Smad, TNFα and NFKB pathways and in CC10B, SSCG were found in MAPK, Smad, TNFα, IFN, FGF and Rho pathways.
Chapter 5, Figure 1. Expression of Smad2, Smad2-P, Smad4 and p21 proteins in primary cervical carcinomas. A tissue-microarray of 117 cervical tumors was stained using immunohistochemistry as described in Materials and Methods (125x magnification). A: Smad2, strong cytoplasmic staining. B: Smad2, moderate cytoplasmic staining. C: Smad2, weak cytoplasmic staining. D: positive nuclear staining of Smad2-P in > 50% of tumor cells. D1: detail of D (400x magnification). E: Smad2-P nuclear staining in < 50% of tumor cells. E1: detail of E (400x magnification). F: Smad4, positive nuclear staining in > 50% of cells. F1: detail of F (400x magnification). G: Smad4, strong cytoplasmic intensity and absent nuclear staining. G1: detail of G (400x magnification). H: Smad4, moderate cytoplasmic intensity. I: Smad4, weak cytoplasmic intensity. J: p21 nuclear staining in > 50% of tumor cells. J1: detail of J (400x magnification). K: p21 nuclear staining in > 0% and < 50% of tumor cells. K1: detail of K (400x magnification). L: absent p21 nuclear staining and L1: detail of L (400x magnification).
Chapter 6, Figure 2. Complex genomic alterations of chromosome 20 (A) and chromosome 8 (B) of SiHa and chromosome 3 of CaSki (C). Below each chromosome ideogram, SNP array data are shown in blue and array CGH data in red. The blue line directly beneath the ideogram depicts LOH, whereas the blue line shifting around the baseline (0) shows copy number retrieved from SNP array data. The dotted blue and red lines represent the confidence intervals for the copy number of the SNP and the CGH array, respectively.
Chapter 6, Figure 3. FISH of metaphase preparations depicting copy number alterations. A) CaSki - 5 chromosomes 5 (centromere 5-blue; TRIO-green; SKP2-red); one isochromosome 5p (yellow arrow), 2 translocations of RP11-1150G22, encompassing TRIO (green arrow) and 2 translocations of RP11-624K2, encompassing SKP2 (red arrow). B) CaSki - 3 chromosomes 8 (centromere 8-red; TUSC3-green) with loss of one copy the BACs encompassing TUSC3, RP11-44L18/184C1 (green arrow). C) HeLa - 4 chromosomes 20 (centromere 20-blue, ZNF-217-red, CYP24A1-green); one isochromosome 20q (yellow arrow).
Chapter 7, 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/SerinA3 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.