Chapter 1
Cancer Immunoediting: Elimination, Equilibrium, and Immune Escape in Solid Tumors

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Abstract Emphasizing the dynamic processes between cancer and host immune system, the initially discovered concept of cancer immunosurveillance has been replaced by the current concept of *cancer immunoediting* consisting of three phases: elimination, equilibrium, and escape. Solid tumors composed of both cancer and host stromal cells are an example how the three phases of cancer immunoediting functionally evolve and how tumor shaped by the host immune system gets finally resistant phenotype. The elimination, equilibrium, and escape have been described in this chapter in details, including the role of immune surveillance, cancer dormancy, disruption of the antigen-presenting machinery, tumor-infiltrating immune cells, resistance to apoptosis, as well as the function of tumor stroma, microvesicles, exosomes, and inflammation.

Keywords Cancer immunoediting · Immunosurveillance · Cancer dormancy · Cancer escape mechanisms

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Abbreviations

AKT	protein kinase B
APCs	antigen-presenting cells
BMP	bone morphogenetic protein
CAFs	cancer-associated fibroblasts
CCR	C-C chemokine receptor
COX	cyclooxygenase
CSCs	cancer stem cells
CSF-1	colony-stimulating factor-1
CTCs	circulating tumor cells
CTLA-4	cytotoxic T lymphocyte-associated antigen-4
CTLs	cytotoxic T lymphocytes
CXCR	C-X-C motif chemokine receptor
DCs	dendritic cells
DTCs	disseminated tumor cells
ECM	extracellular matrix
EMT	epithelial-to-mesenchymal transition
ERK	extracellular signal-regulated kinase
FAK	focal adhesion kinase
FasL	Fas ligand
FGF	fibroblast growth factor
GITR	glucocorticoid-induced tumor necrosis factor receptor
GLI	glioma-associated oncogene homolog
GM-CSF	granulocyte-macrophage colony stimulating factor
Hh	hedgehog signaling
HIF-1α	hypoxia-inducible factor-1α
HLA	human leukocyte antigen
HSP	heat-shock protein
IAPs	inhibitor of apoptosis proteins
IDO	indoleamine 2,3-dioxygenase
IFN	interferon
IGF	insulin-like growth factor
IL	interleukin
ILT	immunoglobulin-like transcript
JAK	Janus kinase
JNK	c-Jun N-terminal kinases
MAPK	mitogen-activated protein kinases
MCP-1	monocyte chemotactic protein-1
M-CSF	macrophage colony stimulating factor
mDCs	mature dendritic cells
MDCs	myeloid dendritic cells
MDSCs	myeloid-derived suppressor cells
MICs	metastasis-initiating cells

MMPs	metalloproteinases
mTOR	mammalian target of rapamycin
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NK	natural killer cells
NKG2D	activating receptor of NK cells
NKT	natural killer T cells
NO	nitric oxide
NOTCH	neurogenic locus notch homolog protein
NR2F1	nuclear receptor subfamily-2 group-F member-1
PD-1	programmed death-1
PDCs	plasmacytoid dendritic cells
PD-L1	programmed death-1 ligand (also called B7-H1)
PGE_2	prostaglandin E_2
PI3K	phosphatidylinositol 3-kinase/phosphatase
RANTES	Regulated on Activation, Normal T-cell Expressed and Secreted (CCL5)
RNS	reactive nitrogen species
ROI/ROS	reactive oxygen intermediates/species
STAT	signal transducer and activator of transcription
TAA	tumor-associated antigen
TAMs	tumor-associated macrophages
TANs	tumor-associated neutrophils
TCR	T-cell receptor
TEMs	tie-2-expressing monocytes/macrophages
TGF-β	transforming growth factor-β
TILs	tumor-infiltrating lymphocytes
TLR	toll-like receptor
TNF-α	tumor necrosis factor-α
Tr1 cells	type 1 regulatory T cells
TRAIL	TNF-related apoptosis-inducing ligand
Tregs	T regulatory cells
uPAR	urokinase plasminogen activator receptor
VEGF	vascular-endothelial growth factor

1.1 Introduction

The idea of cancer immunosurveillance has been built on the hypothesis that cancer cells are recognized as nonself and induce the host response. In fact, cancer cells differ from normal human cells. Neoplastic cells express on their surface antigens, which can be the targets for humoral or cellular response.

Initially, tumor antigens were divided into tumor-specific antigens (TSA) present only on cancer cells and tumor-associated antigens (TAA) found also on noncancer

cells. However, during subsequent investigations, antigens primarily thought as TSA have been found also on normal human cells. Actually, the classification of tumor antigens is based on their molecular structure and origin. Thus, there are differentiation antigens (e.g. tyrosinase or gp-100 in melanoma), overexpression/amplification antigens (e.g. HER-2/neu in ovarian and breast cancer), mutational antigens (e.g. p53, Ras in various cancers), cancer testis antigens (e.g. NY-ESO-1 in ovarian cancer), glycolipid antigens (e.g. MUC-16 in ovarian cancer), oncofetal antigens (e.g. alpha-fetoprotein (AFP) in germ cell tumors, carcinoembryonic antigen (CEA) in colorectal cancer), and viral antigens (e.g. human papilloma virus—HPV in cervical cancer) (reviewed in Liu et al. 2010). At present, more than 1000 human tumor antigens have been described (Cancer Immunome Database). Conceptually, TAAs may be divided into three groups: self-antigens or embryonic antigens overexpressed or respectively aberrantly expressed on cancer cells, self-antigens modified by posttranslational tumor-specific disturbances, and neoantigens originating from mutations, chromosomal aberrations, and viral transformation (Töpfer et al. 2011).

Thus, the intact immune system may recognize TAAs and prevent the development of cancer in a process initially termed immunological surveillance (Burnet 1970). The host response involves both innate and adoptive immune system, which closely cooperate. Generally, the innate immunity is mainly responsible for early detection and elimination of malignant cells, while the adaptive immune system rather controls the tumor progression. However, cancer cells developed variety of strategies to evade the host immune system. They shed surface antigens and downregulate the expression of molecules necessary for interaction with immune cells. They also produce and release factors (cytokines, enzymes) that exert a modifying effect on the host-adaptive immune response or induce the apoptosis of immune cells (Poggi and Zocchi 2006, Whiteside 2006). These host–tumor interactions may or may not result in cancer elimination. When the host-mediated antitumor immunity is stronger, tumor cells are eliminated; otherwise, cancer cells undergo immune escape and grow rapidly (Lin and Karin 2007; Liu et al. 2010).

Emphasizing the dynamic processes between cancer and host immune system, the concept of cancer immunosurveillance (Burnet 1970) has been replaced by the current concept of cancer immunoediting (Dunn et al. 2002) consisting of three phases: elimination, equilibrium, and escape. In the process of elimination, nascent transformed cells are recognized and eradicated by innate and adaptive immune system—if all neoplastic cells are eliminated, cancer immunoediting is finished and consistent with cancer immunosurveillance. If all transformed cells are not eliminated at the beginning, immunological pressure leads to the selection of clones with decreased immunogenicity which successively become resistant to the immune system in the equilibrium phase—tumors are usually still not detectable clinically. Developing tumor creates proinflammatory and immunosuppressive microenvironment leading to the impairment of the host immune function and escape from immunosurveillance resulting in tumor growth and metastases.

1.2 Immunosurveillance of the Host Against Cancer—Elimination

The main effectors of cancer immunosurveillance are natural killer (NK) cells, natural killer T cells (NKT), γδ T cells, cytotoxic T lymphocytes (CTLs), interferon (IFN) γ, perforins, and system Fas/FasL. Their role in the cancer immunosurveillance was firstly confirmed and described in immunologically manipulated mice (reviewed in Kim et al. 2007, Wilczyński and Duechler 2010). Subsequently, clinical findings have supported the conclusions driven from animal studies. The presence of high-density tumor infiltration by NK cells and tumor-infiltrating lymphocytes (TILs) was found in many cancers and correlated with better prognosis and survival in patients with ovarian cancer, breast cancer, lung cancer, oral, esophageal, gastric and colorectal cancer, and malignant melanoma. Moreover, the presence of both tumor-specific cellular (T cells) and humoral (antibodies) response was connected with better prognosis in cancer patients (Whiteside 2010).

Elimination process is initiated when growing tumor cells, and also macrophages and stromal cells present in cancer site release inflammatory cytokines what recruits and activates other innate effector cells like NK, NKT, or γδ T cells. They recognize and destroy neoplastic cells by meaning of perforins, Fas/FasL, TNF-related apoptosis-inducing ligand (TRAIL), and IFN-γ (Smyth et al. 2000). Secreted IFN-γ exerts cytotoxic effects and induces apoptosis of the cancer cells. Necrotic tumor cells release tumor antigens which evolve adaptive response. NK cells promote maturation of dendritic cells (DCs) and their migration to the regional lymph nodes. DCs ingest destroyed tumor cells and their tumor antigens, and after maturation and migration to the regional lymph nodes present the antigens to naïve $CD4^+$ T cells. This presentation generates clonal expansion of tumor-specific $CD4^+$ and $CD8^+$ T cells (CTLs). Tumor-specific CTLs infiltrate tumor site and eliminate the rest of cancer cells expressing tumor antigens (Kim et al. 2007). When all cancer cells are destroyed, the elimination is completed. However, the end may be not so successful.

Dying transformed cells (and also normal human cells) release danger signals like uric acid, heat shock proteins, and extracellular matrix derivatives which may induce proinflammatory response activating innate immune system (Powell and Horton 2005, Shi et al. 2003). Limited inflammatory reaction usually helps eradication of tumor cells, but intense inflammation may promote tumor progression, among others by stimulation of release of immunosuppressive cytokines like interleukin (IL) 10 and transforming growth factor (TGF) β as a feedback loop (Kim et al. 2005). Moreover, genetic instability of cancer cells under host immunologic pressure creates less immunogenic types of cells (Whiteside 2010). Taken together, this weakening of the immune response and decreasing immunogenicity of transformed cells may lead to the next steps of cancer immunoediting—equilibrium and/or escape.

1.3 Cancer Dormancy and Cancer-Immune Equilibrium

Cancer dormancy defined as clinical phenomenon is described by cancer systemic or local recurrence after a long time in a patient who has been considered as completely cured and free of the disease. Such situation has been observed in several tumors, including breast, prostate, renal, thyroid cancer, and melanoma (Uhr and Pantel 2011). The relapse of breast cancer 10–20 years after the primary treatment has been noticed in relatively steady population of 1.5% of patients. It was also shown that circulating tumor cells were present in 36% of breast cancer patients after mastectomy as long as 7–22 years after the surgery (Marches et al. 2006).

Clinical dormancy is probably connected to the existence of several partially overlapping functionally populations of cells called metastasis-initiating cells (MICs), circulating tumor cells (CTCs), disseminated cancer cells (DTCs), and cancer stem cells (CSCs). MICs are a population of either early-stage disseminating or late-stage disseminating cancer cells, usually considered to be in a quiescent or dormant status. MICs are present among CTCs and DTCs residing inside metastatic niche. Quiescence and dormancy are similar states, but when dormancy is a more stable and passive state, quiescence is rather an active and transient program of cell behavior regulated by both occurrence of new or lack of typical signals from the cell environment. CSCs are considered to be a considerable part of a population of quiescent cells in many tumors. Quiescent cells are slow-cycling CSCs possessing increased repopulating ability and capable to resist against a spectrum of unfavorable conditions. Their presence is usually linked to hypoxic, acidic, and necrotic areas of tumor. Quiescent CSCs show expression of genes responsible for activation of both hypoxic (hypoxia-inducible factor-1α—HIF-1α, glucose transporter-1—GLUT1) and dormant (nuclear receptor subfamily-2 group-F member-1—NR2F1, p27) regulatory pathways (reviewed in: De Angelis et al. 2019). Activation of mammalian target for rapamycin (mTOR) pathway is necessary for survival of quiescent CSCs and dormant DTCs (Hen and Barkan 2019).

Increasing evidence seems to support the notion that DTCs quiescent/stem cells are able to disseminate from the early primary tumors as CTCs. This possibility was, between others, raised by the studies suggesting that mammary ductal carcinoma in situ (DCIS) produced disseminated cells. CTCs go through epithelial-to-mesenchymal transition (EMT), enter the bloodstream, and are able to survive in circulation, being the marker of adverse clinical outcome. In the blood, CTCs circulate in the form of cell clusters or circulating tumor microemboli (CTMs) composed of the mixture of cancer cells, platelets, epithelial cells, fibroblasts, and immune cells. The latter contain more cells and therefore have usually a greater metastatic potential than clusters (Liao et al. 2014). Both CTCs and DTCs are cancer cells that either have acquired stemness traits, or alternatively they are true CSCs. Significant similarity in biology of CTCs, DTCs, and CSCs supports this notion. Therefore, metastases may originate from CSC-like cells or true CSCs. Not all CTCs or DTCs are capable of forming micro- and macrometastases, as their metastatic potential is dependent on interactions with premetastatic and metastatic niche. It was found that

about 30% of patients diagnosed as having breast cancer already had micrometastatic disease in bone marrow; however, only 50% of them presented with clinically evident bone metastases in the course of the disease (Marches et al. 2006). There are also "early DTCs" produced by premalignant lesions which are not able to initiate a metastatic growth in target organs due to their insufficient genetic alterations and suppressive signals from the environment. They similarly to normal DTCs enter the state that prevents apoptosis but maintains dormancy (Bragado et al. 2012).

An important observation made in the XIX century by Paget contributes to the contemporary understanding of clinical dormancy. According to it, the metastatic cancer cell potential to survive depends not only on the inherent cell properties, but also on the existence of hostile or hospitable environment in the target organ ("seed and soil" theory) (Ossowski and Aguirre-Ghiso 2010). CTCs disseminated to distant organs cross the vascular barrier more frequently in organs which have more permissive vessels, like bone marrow or lungs. It was shown that breast cancer patients with cells disseminated to the bone marrow had longer disease-free intervals than patients who displayed cell dissemination into other organs. Squamous carcinoma cells were shown to disseminate into multiple organs including lungs, liver, bone marrow, spleen, and lymph nodes; however, only inside lungs and lymph nodes, they developed clinical metastases. Moreover, murine models indicated that cancer cells disseminated to the bone marrow failed to expand unless they were transplanted into irradiated recipients. The connection between environment and behavior of DTCs is further supported by the observation that genes responsible for DTCs quiescence, including *MKK4*, *MKK6*, *KISS1,* and some others, are exclusively activated in the target organs, but not in the primary tumor (Taylor et al. 2008).

In target organs, cancer cells reside in premetastatic niches which are actively created by both cancer cells and local cells recruited from stroma (cancer-associated fibroblasts—CAFs, myeloid-derived suppressor cells—MDSCs) and immune system (tumor-associated macrophages—TAMs, T regulatory cells—Tregs). Organ-specific niches protect dormant DTCs from environmental stressors and drug-dependent toxicity (De Angelis et al. 2019, Klein 2009, Sosa et al. 2014). The premetastatic niche is regulated by several signals from activated cells including growth factors, cytokines, chemokines, and exosomes. This is a hypoxic milieu that promotes survival of cancer cells. Inside niche dormant DTCs are unable to acquire proangiogenic activity and are described by high expression of angiogenesis inhibitors, like angiostatin, endostatin, and trombospondin-1 (reviewed in: Jahanban-Esfahlan et al. 2019). Acidic conditions enhance extracellular matrix (ECM) degradation and inhibit anticancer immune response. TAMs are extensively recruited to premetastatic niche through tumor-derived colony-stimulating factor-1 (CSF-1), vascular-endothelial growth factor (VEGF), CCL2, and CXCL12, and they inhibit host defense against cancer cells via programmed cell death PD-1/PD-L1 checkpoint molecules. Immature DCs and neutrophils being components of premetastatic niche play also important role in deviating of antitumor response (reviewed in: Ingangi et al. 2019). The role of CAFs in promotion of DTCs is practically the same as for CSCs niche [described in the chapter devoted to CSCs]. In

premetastatic niche, DTCs/CSCs with acquired quiescence or dormancy wait until the moment when signals from local environment change the niche into mature metastatic niche. These niche alterations occurring frequently as a result of inflammation could "wake up" DTCs from dormancy, activate angiogenic pathways ("angiogenic switch"), and initiate metastatic growth (reviewed in: Jahanban-Esfahlan et al. 2019; Ingangi et al. 2019). Also others components of metastatic niche are responsible for regulatory switch from quiescence/ dormancy to metastatic growth. Acquisition of stemness, self-renewal, and proliferation properties by DTCs is dependent on EMT. Inhibitors of dormancy mediated by signals from ECM are collagen type I, fibronectin, activation of focal adhesion kinase (FAK)/nonreceptor tyrosine kinase Src/MEK pathway, and aurora kinase-A (AURKA). DTCs are not only responders to signals emitted from metastatic niche components, but also are capable to interfere with them actively. Breast cancer DTCs were shown to stimulate niche stromal cells to release ECM components like periostin and tenascin C, which in turn activated stemness pathways in DTCs mediated by Wnt/β-catenin, NANOG, and octamer-binding transcription factor-4 (Oct4) leading to their metastatic outgrowth (Malanchi et al. 2012, Oskarsson et al. 2014).

There are two forms of dormancy: tumor and cellular dormancy. Tumor dormancy is based on the balance between tumor proliferation and apoptosis dependent mostly on vascular deficit ("angiogenic dormancy") (reviewed in: Hen and Barkan 2019). Angiogenic dormancy is one of the reasons for dormancy of a small micrometastatic tumor (reviewed in: Ossowski and Aguirre-Ghiso 2010). Tumor can slowly proliferate, but is avascular both because of the lack of angiogenic factors expression and the upregulation of angiogenesis inhibitors. Due to this and ongoing apoptosis, the tumor has stable dimensions (Naumov et al. 2006). Escape from angiogenic dormancy triggers the growth of macrometastases which show significantly higher proliferation potential and vascularity. Another cause for dormancy of small micrometastatic tumors is a balance between proliferation and apoptosis dependent on effective immune surveillance of the host against tumor cells. It is called "immune dormancy" (Shiozawa et al. 2013, reviewed in: Hen and Barkan 2019). The problem of tumor immune dormancy is closely connected to the status of cancer-immune equilibrium. The murine studies showed that sarcomas transplanted into T-cell-, IFN-γ-, and IL-12-deficient mice rose vigorously, but were eliminated when retransplanted into immunocompetent wild-type mice. Depletion of innate NK cells or neutralization of the NKG2D and TRAIL pathways had no effect (reviewed in: Teng et al. 2008). Similarly, long-term survivals were demonstrated in mice subjected to adoptive immunotherapy, which however did not eliminate completely transplanted prostate cancers, but instead controlled them in the phase of a small tumor. The equilibrium between T $CD8^+$ cells and small skin tumors was also observed in another murine studies. These findings strictly indicate that adaptive T effectors, IFN-γ, and IL-12 play an important role in controlling tumor growth (reviewed in: Teng et al. 2008). Tumors in cancer-immune equilibrium were slowly proliferating tumors with increased ratio of dying cells and the presence of host immune effectors (reviewed in: Teng et al. 2008). Clinical observations support tumor dormancy hypothesis. It was shown that the late lung cancer remissions

occurred mostly in immunodefective persons, as well as small nondetected tumors transplanted unintentionally with the organs of immunocompetent donors became clinically evident in immunosuppressed recipients (Stewart et al. 1991). The small clinically "silent" tumors were found in the breasts of 39% of women aged 40–50, and in the prostates of 46% of men aged 60–70, subjected to autopsies after death caused by car accidents, but we know that only 1–1.5% of populations at this age have clinically recognizable tumors (Feldman et al. 1986).

The second form of dormancy is called cellular dormancy. Most of dormant solitary cells were isolated from bone marrow of various cancer patients and showed G_0/G_1 arrest with overexpression of p21 and p27. There are plenty of known inducers of cell dormant status, including hypoxia, starvation, components of ECM, cellular stress, activation of signaling pathways, or epigenetic regulation. Cancer cells subjected to metabolic stressors like hypoxia or starvation are prone to dormancy. Disturbances of lipid metabolism, reactive oxygen species, and oxidative DNA damage are inducers of metabolic dormancy of DTCs, while inhibitors comprise mitochondrial dysfunction and activity of mitochondrial serine-beta-lactamase-like protein (LACTB) (reviewed in: Jahanban-Esfahlan et al. 2019).

Unappropriate interactions with ECM of metastatic niche may trigger mechanisms leading to DTC dormancy (reviewed in: Páez et al. 2012, Barkan et al. 2010). Inducers of ECM-dependent dormancy include expression of kisspeptin gene *KISS-1*, urokinase receptor u-PAR, cytokine TGF-β2, E-selectin, SDF-1/CXCR4, WnT5a, insulin growth factor-1 (IGF1)/protein kinase B (AKT) pathway, and GTP-binding RAS-like-3 family molecules (DIRAS3). Expression of *KISS-1* inhibits motility and proliferation of melanoma cells. Receptor u-PAR is one of the key molecules for long-standing survival of cancer cells in bone marrow. Downregulation of urokinase plasminogen activator receptor (uPAR), β1-integrins, FAK, and EGFR reduces proliferative signals from ECM. Prolonged uPAR suppression activates long-lasting dormancy, as was shown by inhibiting of uPAR in squamous cancer cell line. The possible mechanism that triggers dormancy is an uPAR-mediated imbalance between p38 and extracellular signal-regulated kinase (ERK) in the cancer cells, which activates endoplasmic reticulum (ER) stress-like reaction (reviewed in: Ranganathan et al. 2006). Overbalance of the $p38^{high}/ERK^{low}$ status promotes dormancy, while overbalance of $p38^{low}/ERK^{high}$ triggers mitogenesis. Moreover, p38-dependent activation of p53 and inhibition of c-Jun protein, as well as activation of p38/ER chaperone BiP/protein R-like ER kinase (PERK) pathway induces dormancy and quiescence/chemoresistance of cancer cells, respectively (Ranganathan et al. 2006). The interactions between fibronectin and α5β1 integrin were also uPAR-dependent and modulated the ECM functions (reviewed in: Laufs et al. 2006). Impaired signaling through integrins and adhesion signal transducers has been noticed in DTCs of squamous and breast cancers. Disturbed interactions with ECM may also trigger autophagy. The presence of both autophagy and dormancy was confirmed in ovarian cancer cells upon stress conditions (Lu et al. 2008).

Notch and Wnt/β-catenin signaling so important for maintenance of CSCs also control the balance between dormancy and proliferation in DTCs. Cytokine TGF-β2 induces dormancy by protection against cellular adhesion of cancer cells and is

highly expressed in the bone marrow. TGF-β function depends on the type of the target organ, other signals, and the ability of cancer cells to activate alternative cellular pathways to benefit of the proliferative TGF-β activity (Bragado et al. 2012). In the lung, another member of TGF-β family, bone morphogenic protein (BMP) 4, augments dormancy of breast cancer cells. E-selectin and SDF-1/CXCR4 pathway help breast cancer cells to home into premetastatic niche in bone marrow. Similarly, Wnt5a/receptor tyrosine kinase Ror2 (ROR2)/E3 ubiquitin-protein ligase (SIAH2) signaling is engaged in induction of prostate cancer dormancy inside bone marrow. DIRAS3/ERK/AKT signaling induces dormancy via activation of autophagy (Allgayer and Aguirre-Ghiso 2008, Mao et al. 2019). Another recognized inducers of DTCs dormancy are: N-cadherin, Notch, aminopeptidase N (CD13), BMP7, osteonectin (SPARC), sex-determining region-Y box-2 transcription factor (Sox2), TANK-binding kinase-1 (TBK1), p53, and paired related homeobox-1 (PRRX1). Transcription factor HES-1, which induced dormancy but prevented from cell senescence and terminal differentiation, was identified in melanoma cells (Jia et al. 2019, Jiang et al. 2019).

Epigenetic upregulation of NR2F1 nuclear receptor increases expression of NANOG and chromatin repression, which promotes dormancy in breast and prostate cancer. Cells entering dormancy have also epigenetically increased expression of mitogen and stress-activated kinase-1 (MSK1) and transcription factor PCL1 (Sosa et al. 2015, Gawrzak et al. 2018). Another example of epigenetic regulation of DTCs is dormancy-miRNA (called DmiRs). Their transfer inside of exosomes from metastatic niche cells into DTCs promotes quiescence and dormancy, and chemoresistance, and prevents apoptosis. The most known DmiRs are miR-222/223, miR-34a, miR-190, miR-100-5p, miR-200, and miR-125b (Almog et al. 2013, Tiram et al. 2016, Watson et al. 2018).

Regulation of DTCs may also occur via mechanisms of DTCs self-seeding into the primary tumor which usually increases its aggressiveness, and via tumor instigation of distant micrometastases by endocrine factors (reviewed in: Bragado et al. 2012). The latter mechanism is interesting, as osteopontin secreted into the circulation by instigating tumor activates bone marrow-derived cells, which migrate into the dormant tumor and stimulate CAFs to switch dormant cells into proliferative malignant phenotype (reviewed in: Castaño et al. 2011).

1.4 Cancer Escape Mechanisms

1.4.1 Disruption of the Antigen-Presenting Machinery, HLA-G, and Costimulatory Molecules

Tumor-associated antigens originate from self-antigens or embryonic antigens overexpressed or respectively aberrantly expressed on cancer cells, self-antigens modified by posttranslational tumor-specific disturbances, and neoantigens

originating from mutations, chromosomal aberrations, and viral transformation (reviewed in: Töpfer et al. 2011). As most of solid tumors express self- or modified self-antigens, T effectors are unable to recognize them properly due to the central and peripheral tolerance. Peripheral tolerance could be overcome by a process of cross-priming during which DCs, in order to effectively stimulate T effectors, need to encounter antigens associated with "danger signals" (pathogenic-associated molecular patterns—PAMPs) via toll-like receptor (TLR) receptors. Usually, the "danger signals" are derived from microorganisms; however, in cancer, necrotic cells could deliver damage-associated molecular pattern (DAMPs) signals including calreticulin and high-mobility group box-1 protein (HMGB1) (Tesniere et al. 2010, Scaffidi et al. 2002). Low tumor-induced expression of TLR9 receptor on plasmacytoid DCs was observed in head and neck squamous cancer. In colon cancer patients, loss of functional TLR4 resulted in short progression-free survival (Tesniere et al. 2010). DCs which have not been activated by "danger signals" are able to present tumor antigens in the context of MHC molecules; however, this process causes T-cell anergy and apoptosis in a mechanism of cross-tolerance. Observations in cancer patients revealed the presence of soluble forms of human leukocyte antigen (HLA)—sHLA. The data concerning the concentration of sHLA in cancer are not consistent and depend on the tumor type and HLA allotypes. Increased, not changed or decreased, sHLA levels were described in pancreatic, melanoma, and gastric cancers, respectively. sHLA may downregulate activity of CTL and NK cells (reviewed in: Campoli and Ferrone 2008). The mechanism of tumor recognition by T effectors is also disturbed by abnormalities in antigen presentation machinery, including loss or downregulation of HLA class I antigens due to gene mutations, loss of heterozygosity, and disturbed transcriptional regulation (reviewed in: Töpfer et al. 2011). The presence of such mechanisms was confirmed in esophageal, prostate, and lung cancer. Tumors are capable to loose TAAs together with HLA antigens not only spontaneously but in the response to adoptive T $CD8^+$ therapy. Initially effective MART-1/Melan A-targeted adoptive T-cell therapy of HLA-A2-positive melanoma was found to be ineffective in metastases and recurrent tumors due to the loss of expression of MART-1 and HLA-A2 molecules (Dunn et al. 2004). In melanoma and colon cancer, the mutation of β2-microglobulin was observed. Tumors are also characterized by an acquired deficits in antigen peptide transporter (TAP) and low-molecular mass polypeptide (LMP)2 and LMP7 immunoproteasome subunits (Seliger et al. 2000). In melanoma and renal cancer, decreased expression of HLA class I antigen was caused by methylation of TAP-1 and -2 (Seliger 2008). Interferon is capable of upregulation of HLA molecules, but defects in IFN-γ signaling such as mutations of Janus kinases (JAK-1 and -2) may also decrease their expression. In head and neck squamous cancers, downregulation of HLA class I antigen and defective function of members of antigen processing machinery (APM) were correlated with low T $CD8^+$ infiltration, metastases to regional lymph nodes, and poor prognosis (reviewed in: Duray et al. 2010).

Despite these mechanisms, activated NK cells should be able to recognize and kill HLA-negative tumor cells. However, to avoid both CTL and NK-cell-dependent attack, tumor cells express an immunomodulatory nonclassical HLA class I antigen

HLA-G on their surface (reviewed in: Campoli and Ferrone 2008). Epigenetic changes like demethylation or histone acetylation may be responsible for ectopic HLA-G expression on cancer cells. Unfortunately, it seems that host immunosurveillance against tumor accounts for initiating HLA-G, as IFN-producing immune effectors upregulate HLA-G expression. Moreover, tumor-infiltrating immune cells also acquire the HLA-G-positive phenotype, producing strongly immunosuppressive environment inside tumor. Effector cells, by contact with HLA-G both on cancer and on regulatory cells, and via trogocytosis of membrane fragments containing HLA-G from DCs, become inhibited and turned into tolerogenic status (reviewed in Urosevic and Dummer 2008). Several receptors for HLA-G functioning as killing inhibitory receptors (KIRs) have been identified including KIR2DL4/p49, immunoglobulin-like transcript (ILT)-2, and ILT-4, which were found to be expressed on NK cells, T and B lymphocytes, macrophages, and DCs. Therefore, HLA-G is capable not only to inhibit NK cytotoxicity, but also to modulate DCs' activity, followed by inhibition of proliferative T-cell responses (reviewed in Urosevic and Dummer 2008, Sheu and Shih 2007, Pistoia et al. 2007). Through inhibitory ILT-2 receptor, HLA-G disturbs T-cell activation and decreases CD3ζ phosphorylation and IL-2 secretion. In addition to expression of membrane-bound HLA-G, tumors are capable to secrete its soluble form (sHLA-G), having strong systemic immunoregulatory properties. sHLA-G induces Fas-dependent apoptosis of activated T $CD8^+$ CTLs and decreases T $CD4^+$ helper activity. Both membrane-bound and sHLA-G forms induce production of Th2 cytokines, including IL-10, which in this way creates autoenhancing regulatory loop. HLA-G could be also present in exosomes disseminated into the circulation from the tumor (Urosevic and Dummer 2008). Inside established tumors, there are several factors that trigger and support HLA-G expression, including hypoxia (via HIF-1α), chronic inflammation (via nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)), and immunosuppressive IL-10 (reviewed in: Duechler and Wilczyński 2010). Activators of NF-κB transcription factor stimulate also the sHLA-G shedding from cancer cells (Mouillot et al. 2007, Urosevic and Dummer 2003). The presence of HLA-G molecules was confirmed in many cancers, especially these associated with inflammation (Urosevic and Dummer 2008). Concentration of sHLA-G correlates with tumor size. Besides HLA-G, some other nonclassic HLA molecules like HLA-E and HLA-F have been described in tumors, including lung cancers, and their expression indicates bad prognosis. HLA-E exerts additional suppressive signals to lymphocytes through CD94/NKG2A KIR, and HLA-G has stabilizing effect on this molecule (Mouillot et al. 2007, Urosevic and Dummer 2003).

NKG2D (natural killer group 2, member D) receptor is expressed on the surface of NK and some T cells, including activated T $CD8^+$ and some T $CD4^+$, γ/δ T, and NKT cells, respectively. Human NKG2D ligands comprise MHC class I-related chain (MICA and MICB) and UL16-binding protein family (ULBP) members. Ligands for NKG2D are induced on tissues upon inflammation, stress stimuli, and DNA damage during cancer transformation (reviewed in: Campoli and Ferrone 2008). Tumors are capable to disturb the recognition of surface ligands by

NKG2D receptors through several mechanisms (Raulet 2003). Firstly, constant overexpression of NKG2D ligands results in downregulation of NKG2D expression. Moreover, by TGF-β production, cancer can directly downregulate NKG2D expression (Coudert et al. 2005). Soluble MIC molecules released from cancer cells could further disturb CTLs and NK-cell cytotoxicity by downregulation of activating NKG2D receptor, natural cytotoxicity receptor NKp44, and chemokine receptors CCR7 and CXCR1. Model of prostate cancer studied on NKG2D-deficient mice indicated the growth of more aggressive tumors with high expression of NKG2D ligands compared to tumors in wild-type animals. Expression of NKG2D ligands was observed in human colorectal tumors, however varied between different tumor types, and became progressively less frequent in more advanced tumors. High expression correlated with improved survival and NK-cell infiltration (McGilvray et al. 2009).

Costimulatory molecules which transfer positive or negative signals necessary to initiate T-cell responses belong either to classic B7 family (CD80, CD86) or to the family of B7 homologs containing B7-H2, B7-H3, B7-H4, and some others members. Absence of classic costimulatory molecules CD80 and CD86 on the surface of tumor cells produces anergy in T $CD4^+$ lymphocytes recognizing HLA class II antigens (Byrne and Halliday 2003). Recently, B7-H4 homolog, transferring a negative signal for T-cell activation, deserved greater attention, due to its abundance both on the tumor and immune cells in cancer patients (reviewed in: He et al. 2011). B7-H4 molecule by arresting the cell cycle inhibits the activation, proliferation, and clonal expansion of T $CD4^+$ and T $CD8^+$ cells, as well as secretion of stimulatory IL-2 and IFN-γ cytokines. To date, expression of B7-H4 has been confirmed in variety of solid tumors including colon, prostate, lung, gastric, ovarian, pancreatic, uterine cancer, and melanoma (reviewed in: He et al. 2011). Tregs were reported to induce molecules B7-H4 on the surface of DCs and TAMs, where it functioned as an inhibitor of T-cell activation and cytotoxicity (reviewed in: Palucka et al. 2011). Moreover, B7-H4 mediated inhibitory effects on the growth of neutrophils. Besides regulatory effects on the function of immune system, B7-H4 influenced the tumorigenesis by enhancing the proliferation, migration, and invasiveness, and protecting cancer cells from apoptosis, as was shown in ovarian cancer murine model (Cheng et al. 2009). In ovarian cancer, the expression of B7-H4 and the level of soluble B7-H4 correlated with tumor stage, pathological type, and patients' poor prognosis (reviewed in: He et al. 2011). Similarly in breast cancer, the overexpression of B7-H4 was connected with negative receptor status and HER-2/neu positivity. In bladder cancer, B7-H4 promoted EMT and NF-κB signaling pathway. Another group of costimulatory proteins that are functioning as immune response downregulators (so called checkpoint proteins) are cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and programmed cell death protein-1 (PD-1). Both show an immunosuppressive activity and inhibit an excessive immune responses, thus possessing tumor-promoting properties. The CTLA-4 regulates the T-cell priming and activation by binding to CD80 or CD86 molecules. The PD-1 modulates the activity of antigen-primed T effector cells (Gaillard et al. 2016) and acts through binding to one of its ligands (PD-L1; B7-H1) or PD-2 ligand-2 (PD-L2;

B2-DC). PD-1 inhibits T-cell activity by inhibition of the T-cell receptor downstream signaling. It also enhances Treg proliferation and suppressive activity and inhibits both B- and NK-cell activity (Francisco et al. 2009). In the tumor environment, overexpression of PD-L1 could result from activity of oncogenic signaling pathways. Tumors are capable of using the PD-1/PD-L1 pathway to escape from host immunosurveillance. Expression of PD-L1 ligand was described in many cancers, including renal, stomach, bladder, breast, and lung cancers, and was associated with poor prognosis (McDermott and Atkins 2013, Wang et al. 2016). Higher PD-L1 expression was found in malignant tumors compared to benign/borderline tumors (Maine et al. 2014). In advanced ovarian cancer, TILs are abundant population inside tumor; however, they frequently express PD-1 molecule and seem to be functionally incompetent. A soluble form of PD-L1 has been also observed in aggressive renal cancer. Blockade of PD-1/PD-L1 pathway resulted in increased frequency of T CD8 + CD4-CD45RO+ effector memory lymphocytes, B lymphocytes, and MDSCs in tumors (Ribas et al. 2016).

1.4.2 Tumor-Infiltrating Lymphocytes and Immune Escape

Tumor-infiltrating lymphocytes are the heterogeneous population of immune cells, which upon existence of immunoregulatory conditions in tumor environment acquire in most circumstances immunosuppressive or regulatory phenotype and lose at least partially an antitumor effector activity. The composition and activation status of TILs depends on the expression of chemokines and cytokines originating from both cancer and immune cells in tumor environment.

Effector T CD8$^+$ cells in TIL population have been considered to be a good prognostic sign in ovarian cancer (Curiel et al. 2004b); however, there are suggestions that the T CD8$^+$/Tregs ratio could be a better indicator of good prognosis. The presence of T CD8$^+$ effectors capable of recognition of tumor-associated antigens was confirmed in several tumors. In melanoma patients, T CD8$^+$ effectors responsive against melanA/MART-1 cancer antigen were present in peripheral blood and regional lymph nodes, and most of them belonged to population of naïve CD28 + CD45RA^{high} T cells. The rest of melanA/MART-1–reactive T CD8$^+$ effectors belonged to memory T cells, and were abundant especially inside the tumor. Similar observations were done for colorectal cancer (Hamann et al. 1997). However, the antitumor T CD8$^+$-mediated reactivity was not consistently found in peripheral blood of breast cancer patients, and was different compared to T cells isolated from the bone marrow of the same patients (reviewed in: Nagorsen et al. 2003). It seems that irrespective of possessing an effector phenotype T cells might be unresponsive against some tumor antigens *in vivo*, which could result from both suppressive environment and antigen heterogenic immunogenicity. Moreover, distinct regulatory mechanisms are probably engaged in control of TILs' function in different intratumor localizations. In ovarian cancer, increased intraepithelial T CD8$^+$ lymphocyte density was correlated with better prognosis, while the intensity

of stromal T $CD8^+$ infiltrate did not indicate such correlation. It was shown in several tumors including ovarian cancer that many regulatory cytokines present in the tumor and ascites, including IL-10, TGF-β, tumor necrosis factor-α (TNF-α), and VEGF, indicate immunosuppressive actions against effector TILs (Bamias et al. 2008). Inside the tumor, effector TILs are functionally impaired as was indicated by downregulation of CD3ζ chain, decreased proliferation and expression of activation antigens (CD25, CD69, and HLA-DR), and low secretion of stimulatory cytokines, like IL-2, IL-4, and IFN-γ (Chen et al. 1999b, Santin et al. 2001, reviewed in: Frey and Monu 2006). The mechanisms of effector TILs inhibition include also tolerance-inducing plasmacytoid DCs, B7-$H4^+$ macrophages, TAMs, and MDSCs (Piver et al. 1984, Serafini et al. 2004). Expression of galectins by tumor cells is another mechanism of effector TILs' inhibition. Galectins are proteins possessing the same recognition domain as β-galactosides, and involved in cell proliferation, adhesion, migration, apoptosis, and angiogenesis. In human melanoma, the expression of galectin-3, although not consistently observed in every tumor, correlated with apoptosis of TILs. Expression of galectin-1 (Gal-1) in the tumor cells and in its stroma was correlated with malignancy and poor patient's outcome. Expression of galectin-1 in stroma surrounding the cancer cells and in endothelium in tumor-penetrating vessels protects the tumor from host immune reaction. Expression of Gal-1 in head and neck squamous cancer correlated negatively with T effector infiltration, while blockade of Gal-1 activity in melanoma resulted in reduced tumor mass and more abundant T-cell infiltrate (reviewed in: Camby et al. 2006). Another immunoregulatory molecule influencing negatively effector function is indoleamine 2,3-dioxygenase (IDO), which expression was noticed in variety of cancers. Overexpression of IDO in colorectal, ovarian, and endometrial cancers affected the infiltration of tumor with T $CD3^+$, T $CD8^+$, and $CD57^+$ NK cells. In most cases of solid tumors, overexpression of IDO correlated with the abundance of Treg infiltrate, metastases to regional lymph nodes and to distant sites, and short progression-free and overall survival, and was present especially in advanced tumors (reviewed in: Godin-Ethier et al. 2011). However, in different conditions and in certain tumor types, the infiltration of effector TILs may be more vigorous than in most cancers. Tumors showing overexpression of chemokines CCL2, CCL5, CXCL9, and CCL22, activatory cytokines IL-2 and IFNγ, and parallel low concentration of VEGF were infiltrated with significantly increased T-cell number (Bamias et al. 2008). The state of TIL effectors' anergy is not permanent, as cells tested outside the tumor hostile environment presented *in vitro* conditions, expression of activation marker (HLA-DR), and costimulatory molecules (CD28, CD80, and CD86) and indicated cytotoxicity against cultured ovarian cancer cells (Santin et al. 2001, Freedman et al. 2004). Not only TILs but also peripheral blood lymphocytes (PBLs) may be functionally impaired in cancer patients. The functional impairment and downregulation of JAK3, signal transducer and activator of transcription (STAT) 3, and CD3-zeta signaling molecules in PBLs of ovarian cancer patients were noted (Klink et al. 2012a).

$CD4^+CD25^+Foxp3^+$ T regulatory cells are one of the most important cells promoting tumor escape and indicating an unfavorable prognosis for cancer patients.

An increase in the number of Tregs in peripheral blood, lymph nodes, and spleen of cancer patients has been repetitively noted (reviewed in: Wilczynski et al. 2008). Consistent with these observations, the patients with gastric and esophageal cancers showed increased numbers of circulating peripheral blood natural Tregs. Population of Tregs-infiltrating tumors was also present inside tumors themselves and was more abundant in advanced tumors compared to early-stage disease, with it being a poor outcome predictor in certain tumors (Curiel et al. 2004). Accumulation of Tregs was observed in variety of solid tumors including lung, pancreatic, breast, liver, ovarian, gastrointestinal, and head and neck cancers (reviewed in: Töpfer et al. 2011). It seems that expansion of Tregs includes both population of natural circulating and local induced Tregs (reviewed in: Janikashvili et al. 2011). Tumor-derived TGF-β correlated with the intensity of Tregs' infiltrate in gastric cancer and was the inducer of local population of Tregs from naïve T $CD4^+CD25^-$ cells. In variety of tumors including breast or gastric cancer and melanoma, the Tregs' recruitment to the tumor site is regulated by the CCR4-dependent attraction induced by CCL22 or CCL17 secreted by the cancer cells, macrophages, and DCs (reviewed in: Amedei et al. 2012, Janikashvili et al. 2011). The way of attraction may influence the activation status of Tregs. One of the most important factors of Tregs promotion is expression of IDO by both cancer cells and myeloid DCs. The expression of IDO is associated with poor clinical outcome in ovarian cancer (Cannon et al. 2011, Sharma et al. 2009, Inaba et al. 2009). Similarly, tumors secreting increased levels of TGF-β were characterized by increased Tregs infiltrate and disturbed T $CD8^+$ and T $CD4^+CD25^-$effector activity evidenced by a low secretion of IL-2, IFN-γ, and TNF-α (Curiel et al. 2004). The potent sources of TGF-β are also intratumoral immature DCs. TGF-β induces in T cells an intracellular Smad-2 and -3 signaling pathway and STAT3 and STAT5 activation which result in switch into Tregs phenotype. Another regulators of Tregs expansion are mechanisms engaging interactions of T-cell CTLA-4 and glucocorticoid-induced tumor necrosis factor receptor (GITR) with corresponding ligands on DCs, as well as interactions between PD-1 on T cells with B7-H1 expressed on DCs and TAMs (reviewed in: Janikashvili et al. 2011). Immunoregulatory Tregs could effectively inhibit host defense against cancer based on cytotoxic effectors like $CD8^+$ lymphocytes, NK, NKT cells, and antigen-specific T $CD4^+CD25^-$ lymphocytes, as well as could reversely block maturation of DCs. *In vitro* studies on cultured human cells revealed that by blocking NKG2D receptor on NK cells with membrane-bound TGF-β, Tregs were capable of blocking NK-cell activity and IFN-γ secretion. Both low number of circulating NK cells and downregulation of NKG2D expression on NK cells were poor prognostic factors in colon cancer patients (Ghiringhelli et al. 2005a). It was also presented that $CCR4^+$ Tregs utilized galectin-1 to inactivate NK cells in metastasizing breast cancer. Tregs could also upregulate expression of B7-H3 and B7-H4 immunosuppressive molecules on DCs, which contributed to DC-mediated inhibition of T effectors activity (reviewed in: Janikashvili et al. 2011). Murine studies indicated that Tregs were capable to impair the expression of costimulatory CD80, CD86, and CD40 molecules on DCs and secretion of proinflammatory IL-12 and TNF-α molecules. Tregs-mediated suppression of antigen-presenting function of DCs is dependent on TGF-β

and IL-10 secretion. Tregs closely cooperate with MDSCs to promote tumor growth; however, they might have different roles. Tregs could protect tumors in early stages of proliferation and metastases when host antitumor defense is still effective, while MDSCs augment tumor progression and induce systemic suppression (reviewed in: Biragyn and Longo 2012). GITR has been discovered due to its role in reversing immunosuppressive effects of Tregs in mice. Expression of GITR in humans was confirmed on Tregs and at low levels on T $CD4^+$ and T $CD8^+$ cells, and its action is mediated by combining to the GITR-ligand (GITR-L). It was shown that gastrointestinal tumor cell lines indicated the expression of GITR-L. The GITR/GITR-L signaling downregulated the CD40, CD54, and epithelial cell adhesion molecule (EpCAM), as well as induced TGF-β secretion by tumor cells. Constitutive expression of GITR-L by cancer cells diminished antitumor NK-cell activity (Baltz et al. 2007). Independently on their detrimental effects on tumor host immunity, Tregs exert in some circumstances positive functions. Tregs triggered and stimulated by recognition of gut bacteria could reduce risk of gastrointestinal tumors through downregulation of inflammation (Erdman et al. 2010). In familial ovarian cancer, the observation that high Tregs density correlated with better prognosis was consistent with clinical observation that patients with familial ovarian cancer and carriers of BRCA mutations have better outcome, although their tumors are usually more aggressive (Mhawech-Fauceglia et al. 2013).

Tr1 T lymphocytes represent another group of regulatory IL-10-producing cells generated upon immature DC stimulation. The detailed profile of secreted cytokines specific for Tr1 cells includes IL-10, TGF-β, and trace amounts of IFNγ. The possible role of type 1 regulatory T cells (Tr1 cells) for human pathology and unfavorable outcome was confirmed in studies of different types of tumors (Moore et al. 2001). It was shown that Tr1 cells primed by cyclooxygenase (COX) 2 were associated with inhibition of DC maturation and contributed to increased growth of head and neck squamous cancer. Moreover, murine model revealed that IL-10-knockout or Tr1-depleted mice showed improved antitumor immunity. The population of regulatory T cells with similarity to Tr1 cells' profile of secreted cytokines makes Th3 cells. In addition to TGF-β and IL-10, they are able to produce IL-4 (MacDonald 1998). The importance of Tr1/Th3 infiltrate for progression of B16 melanoma was documented in murine studies, where inoculation of melanoma cells into mice resulted in expansion of Tr1/Th3 cells inhibiting cytotoxic reactions from T $CD8^+$ and NK cells (Seo et al. 2002).

T $CD4^+$ Th17 cells are the next population of lymphocytes engaged in immunoregulatory mechanisms existing inside the tumor, which upon stimulation by IL-23 produce IL-17 (Castellino and Germain 2006, Steinman 2007, Bi et al. 2007). In murine model, Th17 cells promoted growth of transplanted cervical cancers into the nude mice. Increased number of Th17 lymphocytes was noted in several solid tumors, including melanoma, breast, colon, and hepatocellular carcinoma, with some of them having a bad prognostic factor. Similarly, increased number of peripheral blood Th17 lymphocytes was observed in gastric cancer patients. In most advanced cases, the Th17 cells were seen abundantly in tumor-draining lymph nodes (reviewed in: Amedei et al. 2012). High numbers of Th17 cells have

been identified among ovarian tumor TILs, and IL-17 was consistently detectable in both serum and ascites of epithelial ovarian cancer (EOC) patients (Su et al. 2010). Tumor cells, cancer-associated fibroblasts, TAMs, T cells, and antigen-presenting cells (APCs) produce proinflammatory cytokines (IL-1β, IL-6, IL-23, TNF-α) that facilitate the expansion of Th17 cells in tumor environment. The Th17 upregulation in the mouse model of ovarian cancer depended on the secretion of TNF-α by cancer cells. Consistent with this observation, treatment with anti-TNF antibody reduced serum IL-17 levels in EOC patients. Chemoattraction of Th17 cells by both tumor- and CAFs-derived chemokines monocyte chemotactic protein-1 (MCP-1 also CCL2) and Regulated on Activation, Normal T-cell Expressed and Secreted (RANTES—CCL5) was demonstrated. TAMs could participate in Th17 expansion by production of proinflammatory cytokines. The role of Th17 cells for enhancement of tumor growth is probably based on their vasculogenic abilities (Numasaki et al. 2003, reviewed in: Amedei et al. 2012). However, the results of studies concerning the role of Th17 cells and IL-17 have been inconclusive, as have indicated its functional ambiguity both for promotion and rejection of tumors (Langowski et al. 2006, Numasaki et al. 2003, Bettelli et al. 2006). It was shown that Th17 cells secreting IFN-γ and IL-17 were able to upregulate CXCL9 and CXCL10 chemokines, thus leading to chemoattraction of NK and T cytotoxic cells (Kryczek et al. 2009). The protective role of Th17 cells against tumor progression was observed in ovarian and prostate cancers, and the number of Th17 cells increased in patients treated because of breast cancer and metastatic melanoma with monoclonal antibodies (reviewed in: Amedei et al. 2012).

Natural killer T lymphocytes express both T-cell receptor and receptors characteristic for NK cells. Two subpopulations of NKT cells dependent on the presence (NKT I) or absence (NKT II) of the invariant Vα14Jα18 T cell receptor (TCR) Vβ chain have been recognized, and it was found that while NKT I cells mediate tumor rejection, the NKT II cells allow for its growth (reviewed in: Terabe and Berzofsky 2008). Both number of NKT I cells and their responsiveness to α-galactosylceramide (α-GalCer-specific activator of NKT cells) stimulation were decreased in solid cancers, as well as their proliferative activity and capability of IFN-γ production (reviewed in: Terabe and Berzofsky 2008). Low circulating number of NKT I cells in head and neck squamous cancer was an independent predictor of poor survival, while high $V\alpha24^+$ NKT I cell infiltration in colorectal cancers was correlated with favorable prognosis of progression-free and overall survival. The role of NKT II cells for tumor promotion was confirmed in murine studies of renal cell cancer and fibrosarcoma models; however, studies indicated that the extent of suppression revealed by NKT II cells may vary between different tumors (Crowe et al. 2002). The NKT cells inside tumors are engaged in a couple of regulatory networks. One of them counteracts the functions of NKT I and NKT II cells probably by direct cell–cell interactions or through an intermediary anergic plasmacytoid DCs. In another network presented in murine model, Tregs seemed to reduce the number, proliferative response, and cytokine secretion of NKT I cells. Activated NKT I cells were shown to produce IFN-γ and IL-2 which together with IL-12 secreted by APCs activated NK cells (Eberl and MacDonald 2000). They also induced maturation of

DCs by upregulation of costimulatory molecules, expression of class II MHC, and IL-12 secretion. On the other hand, myeloid DCs (MDCs) in the peripheral blood of melanoma and renal cancer induced NKT I cells reversible dysfunction mediated by TGF-β and IL-10. The suppressive NKT II cells activity is based on function of IL-13 which promotes the expansion of M2-type macrophages and stimulates IL-13 receptor-positive Gr-1^{+}CD11b^{+}MDSC cells to inhibit T CD8^{+} effectors by secretion of TGF-β (Terabe and Berzofsky 2008).

Lymphocytes B are a heterogeneous population of cells which, according to the recent studies, possess the protumoral regulatory activity. They could mediate suppression of immune reactions, as the loss or inactivation of B lymphocytes reduced the number of Tregs and MDSCs (reviewed in: Biragyn and Longo 2012). Production of immunoglobulins by B cells initiates creation of immune complexes which could initiate FcR- and complement-dependent chronic inflammation promoting cancer (de Visser et al. 2005). Tumor-infiltrating B cells produce lymphotoxin α/β, which through activation of STAT3 in prostate cancer cells sustains their growth. Moreover, immunoglobulins could function as a carrier for immunosuppressive TGF-β. Lymphocytes B stimulate also M2-type polarization of macrophages by IL-10 and induce T-cell anergy, especially in the case of advanced tumors. They can also influence the Th1/Th2 balance (reviewed in: Biragyn and Longo 2012). B-cell-deficient mice were shown to be resistant to syngeneic tumors including colon carcinoma and some types of melanoma, whereas partial B-cell depletion was correlated with reduced tumor growth in mouse model of colorectal cancer (reviewed in: DeNardo et al. 2010). However, it seems that the precise role of B cells depends on B-cell subpopulation studied, the tumor type, and particular immune situation inside, as is syngeneic mouse melanoma model depletion of B cells' enhanced tumor growth and metastases (Schreiber et al. 2000). Some populations of B lymphocytes possessing immunoregulatory properties and called Bregs have been described. The possible role for Bregs in cancer is suggested by the studies on breast cancer-producing lung metastases. Bregs engaged in this pathology are characterized by a phenotype similar to immature B2 cells with high CD25, CD81, and B7-H1 expression. Their suppressive activity is based not on IL-10 secretion, but instead on generation of TGF-β-producing Tregs. Breg-like cells have been generated *in vitro* from B cells treated with conditioned media from breast, ovarian, and colon cancer cell cultures (Olkhanud et al. 2011).

1.4.3 Immunoregulatory Function of Tumor-Associated Myeloid Cells (TAMCs)

Tumor-associated myeloid cells (TAMCs) constitute the heterogenic population of cells of common myeloid lineage and include at least four cell subpopulations: MDSCs, TAMs, tumor-associated neutrophils (TANs), and the angiogenic

monocytes/macrophages expressing endothelial kinase-2 (Tie-2) called TEMs (reviewed in: Sica et al. 2012).

Myeloid-derived suppressor cells characterized in mice by $CD11b^+/Gr\text{-}1^+$ phenotype (monocytic $Ly6C^+$ or granulocytic $Ly6G^+$) are a multifunctional population of marrow-derived cells involved in the immunosuppression of host immune responses against cancer, which function links the mechanisms of chronic inflammation and tumor progression (Bennaceur et al. 2009). In humans, MDSCs are characterized as $CD14^-CD11b^+$ cells or alternatively $CD33^+$ cells lacking the expression of mature myeloid or lymphoid markers (Serafini et al. 2006; Nagaraj and Gabrilovich 2008). It seems however that in humans, precise phenotype of the MDSCs depends on the tumor type (reviewed in: Sica et al. 2012). Similarly like in mice, human MDSCs could also belong to either monocytic or granulocytic line. Monocytic M-MDSCs are able to differentiate into macrophages and mature DCs, and exert their regulatory effects via nitric oxide (NO), suppressory cytokines, and arginase 1 (ARG1) activity. Granulocytic G-MDSCs suppress immune responses via direct cell-to-cell contact and reactive oxygen intermediates (ROI)/reactive nitrogen species (RNS) (reviewed in: Sica et al. 2012). MDSC cells are scarcely represented in spleen and almost absent in the lymphatic nodes; however, in the presence of tumor, they expand and start to be abundant in spleen, lymph nodes, tumor sites, and malignant ascites (Serafini et al. 2006; Nagaraj and Gabrilovich 2008). Receptor CCR2, C5a component of the complement, and proinflammatory S-100 proteins are responsible for chemoattraction of MDSCs into tumor (reviewed in: Sica et al. 2012). This unique cell population possesses the common feature of suppressing in both antigen-specific and nonspecific manner of host antitumor responses mediated by T $CD8^+$ CTLs, NK cells, and NKT cells, as well as of blocking DCs' maturation (Serafini et al. 2006). The pleiotropic effects of MDSCs are mediated through production of ARG1 and ROI/RNS (Serafini et al. 2006; Rodriguez and Ochoa 2006; Kusmartsev and Gabrilovich 2006), inhibition of T $CD8^+$ CTLs, induction of T $CD4^+CD25^+Foxp3^+$ Tregs, and promotion of Th2-biased environment by secretion of IL-10 and blocking macrophage-derived IL-12 production (Sinha et al. 2007a). The tumor cells could participate in differentiation of MDSCs by secretion of granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), IL-6, VEGF, and prostaglandin E_2 (PGE_2) (Gabrilovich and Nagaraj 2009). Cytokines IL-1β, IL-6, and PGE_2 increase accumulation and suppressive activity of MDSCs (Bunt et al. 2006; Sinha et al. 2007b). In the tumor site, the main activity of MDSCs is based on nonspecific inhibition of immune effectors mediated by NO and ARG1 production. NO inhibits T effectors by interfering with intracellular JAK3 and STAT5 pathways, induction of T-cell apoptosis, and downregulation of MHC class II expression. ARG1 activity depletes arginine and causes the translational blockade of CD3 ζ chain. In the peripheral lymphoid organs, MDSCs inhibit T cell by production of ROI/RNS during the direct cell-to-cell contact (Nagaraj and Gabrilovich 2008). Action of MDSCs against T $CD8^+$ CTLs is probably based on modification of TCR-binding activity caused by peroxynitrite activity (Nagaraj et al. 2007). A correlation between high peroxynitrite concentration and immunosuppression was demonstrated in

various cancers including pancreatic, head and neck, breast cancers, mesothelioma, and melanoma (Nagaraj and Gabrilovich 2008). MDSC-inhibited T $CD8^+$ cells are unable to secrete IFN-γ and IL-2, and to kill the target cells (Kusmartsev et al. 2005). It was also found that MDSCs inhibited T cells by depletion of cysteine which is essential for T-cell activation. Moreover, they were capable to downregulate CD62L selectin expression on T cells, thus reducing their migration into regional lymph nodes (reviewed in: Srivastava et al. 2012). Myeloid-derived suppressor cells are also capable of inducing tumor mutations and thus augmenting the tumor metastatic potential (Bennaceur et al. 2009). By production of IL-10, MDSCs could also skew the function of TAMs into protumoral M2-type activity (Sinha et al. 2007a). They promote the formation of a new blood vessel by expressing metalloproteinases and increasing the bioavailability of VEGF (Murdoch et al. 2008). Circulating MDSCs may differentiate in hypoxic tumor environment into Gr1-$F4/80^+$ macrophages (Kusmartsev and Gabrilovich 2006). The expansion and functional activation of MDSCs are regulated by NF-κB, as IL-1β signaling crucial for recruitment of MDSCs into gastric cancer was found to be NF-κB-dependent. The STAT system also regulates MDSCs' function. STAT1 is responsible for MDSCs' interferon-dependent activation, and STAT5 is engaged in MDSCs' survival (reviewed in: Sica et al. 2012).

Macrophages constitute one of the major immune cell population responsible for both tumor rejection and promotion (Ostrand-Rosenberg 2008; Sica et al. 2008; Siveen and Kuttan 2009), but their function is determined by the way they are activated. There are two groups of macrophages: one are tissue-resident macrophages derived from embryonic yolk sac, and second are infiltrating macrophages recruited from bone marrow monocytes. In the tumor microenvironment, they are converted into TAMs (tumor-associated macrophages). The presence of IFNγ, GM-CSF, TNF-α, lipopolysaccharide (LPS), or other Toll-like receptors ligands shifts their activity into the so-called M1 profile, while stimulation by IL-4, IL-10, IL-13, or TGF-β results in the M2 profile (Mills et al. 2000). Tumor MDSCs in murine breast cancer model were shown to contribute to M2 switch of TAMs, similarly like cancer-associated fibroblasts (reviewed in: Sica et al. 2012). It was demonstrated that T $CD4^+$ lymphocytes by secretion of IL-4 and IL-13 potentiated the metastasis capabilities of adenocarcinoma by stimulation of M2-type TAMs (DeNardo et al. 2010). Lymphocyte B also participates in skewing activity of TAMs into M2 phenotype by stimulating Fcγ receptors on resident myeloid cells. Several additional signals switching the differentiation of macrophages into M2-type were identified, including hormones, growth factors, and bacterial products (reviewed in: Sica et al. 2012). However, it seems that polarization into M1 and M2 phenotypes is somehow artificial and represents the extremal differentiation status, while many cells indicate a functions being a mixture of M1/M2 phenotypes with balance slightly pushed toward M1 or M2 type (Mantovani et al. 2002). Different signals present in tumor environment could be the source of heterogeneous activation contributing to different patterns of gene activation in macrophages. Macrophages with mixture of both M1 and M2 phenotypes have been identified in tumors (reviewed in: Sica et al. 2012). Macrophages of M1 type could effectively

destroy tumor cells through production of Th1 cytokines and stimulation of T $CD8^+$ CTLs (Ostrand-Rosenberg 2008). Conversely, macrophages of M2 type produce mainly IL-6, IL-10, TGF-β, and VEGF and have poor APC abilities. M2-type macrophages regulate inflammation into chronic phase and stimulate tissue healing and remodeling as well as angiogenesis. This cell subset constitutes the vast majority of TAMs, which play a discreditable role in tumor progression (Ostrand-Rosenberg 2008, Sica et al. 2008). Mouse studies confirmed the importance of M2-type TAMs in tumor progression. Src homology-2 containing inositol-5′-phosphatase-1 (SHIP1)-deficient mice, which show spontaneous generation of M2-shifted macrophages, demonstrate increased growth of transplanted tumors. And in contrast, p50 NF-κB-deficient mice, which are unable to mount M2 polarization, show resistance to transplantable tumors. It was shown that most aggressively growing tumors were infiltrated by large numbers of TAMs. Recruitment of macrophages into tumors is regulated by Th2 cytokines, chemokines (Sica et al. 2008, Mantovani et al. 2006, Ben-Baruch 2006), urokinase plasminogen activator (uPa), microbial defensins, and hypoxia. Some of the attractants are universal for many tumors, while some are exclusively secreted by certain tumor types, for instance, uPa and defensins in prostate and gastric cancer, respectively (reviewed in: Sica et al. 2012). CSF-1 and TGF-β are major cytokines that are believed to play important role for recruitment of macrophages into the tumors. Both of them are expressed constitutively on the surface of solid tumors (Wojtowicz-Praga 2003) correlated with intensity of TAMs infiltrate and poor prognosis for the patients (Sapi 2004). The chemokines CCL2 (MCP-1) and CCL5 (Regulated on Activation, Normal T-cell Expressed and Secreted—RANTES) were found to be expressed predominantly by the solid tumors (Zhou et al. 2004). Their overexpression correlated with intratumor TAMs' content as well as with bad survival ratio. They were also shown to regulate migration of peripheral blood monocytes into the tumor. Upon the tumor-derived M-CSF, attracted monocytes differentiate to macrophages. High M-CSF production correlates with poor outcome in ovarian, breast, and endometrial cancers (reviewed in: Allavena and Mantovani 2012). The chronic inflammation recognized as an important component of carcinogenesis is regulated by TAMs, which triggered by tumor-derived inflammatory cytokines (TNF-α), and components of necrotic cancer tissues secrete in turn inflammatory chemokines (CCL2, CXCL1, CXCL8, CXCL12), IL-6, and TNF-α generating self-enhancing loop. IL-6 secreted by TAMs plays important role in stimulation of both cancer and stromal cells. It activates STAT3 pathway in tumor cells making them more proliferative and apoptosis resistant (reviewed in: Allavena and Mantovani 2012). The number of TAMs correlates with advancement of the tumor. High-grade ovarian tumors were characterized by more abundant $CD68^+$ and $CD163^+$ TAMs populations, and a correlation between $CD68^+$ macrophages and Tregs was noted, suggesting the cooperation between both populations existing on the regulatory level (Mhawech-Fauceglia et al. 2013). TAMs are also the most abundant mononuclear cell population in the ascites of ovarian cancer patients, where they contribute to suppression of T effector cells by secretion of IL-10 and TGF-β (Gordon and Freedman 2006). A hypoxic environment inside solid tumors is another attractant for macrophages. Anaerobic conditions increase expression of

endothelin-2 (ET-2) and VEGF, as well as chemokine CXCL12 and receptor CXCR4, which become a stimulus for macrophage recruitment into hypoxic areas of the tumor (Raghunand et al. 2003). Adaptation of TAMs to a hypoxic environment depends on function of HIF-1α, which not only helps TAMs to function in anaerobic environment, but also contributes to proangiogenic and prometastatic TAMs activity. Clinical studies seem to confirm that there is an enhancement of invasiveness and peritoneal metastatic activity in ovarian cancer under hypoxic conditions. Tumor-associated macrophages secrete Th2 cytokines, enhance intratumor angiogenesis (by VEGF, TGF-β, and fibroblast growth factor—FGF), and augment extracellular matrix remodeling (by metalloproteinases—MMPs), thus promoting tumor growth and intravasation of cancer cells into blood vessels and resulting in increased tumor metastatic potential (Ostrand-Rosenberg 2008, Sica et al. 2008, reviewed in: Wilczyński and Duechler 2010). TAMs also secrete some specific molecules like semaphorin 4D (Sema4D) and growth-arrest specific-6 (Gas6) which promote cancer neoangiogenesis and proliferation. Subsets of TAMs not completely biased toward M2-type activity may secrete some amounts of Th1 cytokines, for instance TNF-α. Although TNF-α is considered to be an antitumor cytokine, it has also some protumor activities. It might contribute to DNA damage, induce angiogenic factors, and act as a growth factor for cancer cells (Balkwill 2002). Investigations performed on ovarian cancer indicated that TAMs were also able to inhibit host T effectors by expression of B7-H4 costimulatory molecule, which was identified as a negative regulator of T-cell activation. Tumor-associated macrophages could also exert immunoregulatory effects by secretion of NO and ROI. Investigations have confirmed that tumors compared to normal tissues are characterized by both higher expression of nitric oxide synthase (NOS) and production of ROI, and that their activity is related to TAMs (Malmberg 2004, MacMicking et al. 1997, Bogdan 2001, Thomsen and Miles 1998). Defective M1-type functions showed by TAMs are probably caused by disturbed activation of NF-κB in response to proinflammatory stimuli present in advanced tumors, including TNF-α. Factor NF-κB is responsible for regulation of transcription of many genes including those for cytokines, chemokines, and antiapoptotic molecules (Ostrand-Rosenberg 2008). The STAT signaling molecules also play an important role for TAMs function. STAT3 and STAT6 are activated in M2-type TAMs, whereas STAT1 in M1-type TAMs respectively (reviewed in: Sica et al. 2012).

Tumor-associated neutrophils are a population of $CD11b^{+}Ly6G^{+}$ cells which have longer life-span than typical neutrophils, due to hypoxia and IL-1 present in tumor environment, and are able to mediate chronic inflammation and angiogenesis. Despite the phenotypic similarity and partly overlapping markers, TANs and granulocytic MDSCs seem to be the distinct cell populations. The recruitment of TANs depends on the CXCL8 (IL-8) and TGF-β activity (reviewed in: Sica et al. 2012). The presence of TANs was verified and confirmed in several tumors, including kidney, breast, colon, and lung cancers, and consistently correlated to poor prognosis in renal, breast, and lung cancer (reviewed in: Sica et al. 2012). TANs contribute to tumor growth by promoting the angiogenesis, proliferation, and metastases, and on contrary, their depletion inhibits the tumor growth. It seems that two subpopulations

of TANs exist in the tumor environment: N1-type TANs capable of tumor rejection by TGF-β and ROI function, and N2-type TANs which are TGF-β-negative and promote tumorigenesis. It was suggested that N1-type TANs are fully activated neutrophils, whereas N2-type TANs are immature ones (reviewed in: Sica et al. 2012). TANs could secrete hepatocyte growth factor (HGF) and oncostatin which augmented invasiveness of cancer cells and upregulated expression of CXCR4 (reviewed in: Reiman et al. 2007). Upon activation, neutrophils secrete fibers composed from proteins and chromatin, called neutrophil extracellular trap (NET), and used for entrapment and killing microbes and activation of DCs and T cells. The presence of NET was observed in TANs' infiltrating Ewing sarcoma, in patients with early relapse of the disease. The tumor-promoting role of NET could be an activation of tolerogenic DCs or degradation of extracellular matrix to augment metastases (Berger-Achituv et al. 2013). The peripheral blood neutrophils could also participate in tumor growth promotion, as IL-8 secreted by neutrophils together with upregulation of CD11b/CD18 on their surface facilitated melanoma cell arrest on endothelium and tumor cell extravasation (Dong and Robertson 2009). Moreover, *in vitro* studies have shown that ovarian cancer cells could participate in potentiation of peripheral blood neutrophils inflammatory responses (enhancement of reactive oxygen species (ROS) formation) by the direct cell-to-cell contact (Klink et al. 2008). The activation of ovarian cancer patients' neutrophils by ovarian cancer cells was dependent on the interaction of HspA1A originating from ovarian cancer cells, with TLR2 and TLR4 expressed on the surface of neutrophils (Klink et al. 2012).

Tie-2-expressing monocytes/macrophages are a population of $CD11b^{+}/Gr1^{low/-}/Tie\text{-}2^{+}$ cells which express endothelial kinase-2 (Tie-2) receptor for angiopoietin. They originate from peripheral blood $Tie\text{-}2^{+}$ monocytes which have been recruited to the tumor by hypoxia-triggered chemokine CXCL12 and Ang-2. Moreover, it seems that CXCR4 may be engaged in this recruitment as CXCR4 blockade was connected with significant reduction of TEMs' infiltrate in breast tumors. Engagement of Ang-2 is not restricted to chemotactic attraction of TEMs, but also regulates tumor promotion by increase of IL-10 secretion by TEMs, stimulation of Tregs, and inhibition of M1-type TAM function (reviewed in: Sica et al. 2012). TEMs are related to M2-type TAMs and have however a more M2-skewed functional signature, with pronounced expression of ARG1, scavenger receptors, and lowered expression of IL-1β, COX2, IL-12, TNF-α, and iNOS. They also express proangiogenic molecules, like VEGF and MMPs (reviewed in: Sica et al. 2012). TEMs play a crucial role in tumor angiogenesis. They are seen mainly in the hypoxic areas of the tumor in the proximity of the vessels. Mouse studies confirmed that ablation of $Tie\text{-}2^{+}$ macrophages inside the breast tumors and gliomas resulted in reduction of tumor vasculature and mass, whereas injection of tumor cells together with TEMs significantly augmented tumor vascularization.

1.4.4 Dendritic Cells as Tumor Growth Enhancers

Dendritic cells are professional antigen-presenting cells of myeloid or plasmacytoid origin (Colonna and Liu 2004; O'Neill and Bhardwaj 2004). MDCs are characterized by $CD11c^{+}CD33^{+}CD45RA^{-}CD123^{-}$, whereas plasmacytoid DCs (PDCs) by CD11c-$CD4^{+}CD45RA^{+}CD123^{+}$ phenotype, respectively. PDCs show exclusively expression of TLR7 and TLR9, as well as IFN secretion upon viral stimulation. On the contrary, MDCs indicate the expression of a broad spectrum of TLRs, excluding TLR7 and -9, and are not capable to secrete IFN on viral challenge. Dependent on the environmental factors and signals of activation, DCs are able to stimulate either Th2 or Th1 responses. Inside the tumor environment, DCs acquire regulatory properties (reviewed in: Fricke and Gabrilovich 2006; Palucka et al. 2011). Presence of competent mature DCs (mDCs) is very rare in the tumors, which was confirmed in ovarian, prostate, breast, and renal cancers (reviewed in: Fricke and Gabrilovich 2006). If present, they occupy the peritumoral tissues. On the contrary, progressive tumors usually contain DCs having immature $CD4^{-}CD8^{-}$ phenotype (iDCs). Opposite to mature DCs, these cells indicate protolerogenic functions and are unable to effectively stimulate cytotoxic responses (Liu et al. 2005). Moreover, they are able to inhibit tumor-specific T $CD8^{+}$ cytotoxic responses even in chemotherapy pretreated mice, by capturing $CD8^{+}$ CTLs into DCs reach areas of the tumor. There are tumor-derived immunoregulatory factors that are responsible for defective maturation and differentiation of DCs. Lack of immunostimulatory IL-12 and IFN-γ in tumors creates an environment which blocks DCs' maturation (reviewed in: Fricke and Gabrilovich 2006). Tumor environment also contains many other cytokines and immunoregulatory factors that modulate DC function, and among them are cytokines such as VEGF, IL-10, IL-6, TGF-β, and PGE_2, factors like IDO and ROI, and finally tumor antigens and metabolites (reviewed in: Bennaceur et al. 2009). The meaning of VEGF for DCs function was shown in murine studies, where use of VEGF-neutralizing antibody stimulated DCs' differentiation and raised the number of mDCs, while in the presence of VEGF, the DCs showed disturbed antigen-presentation capacity. Murine studies found the presence of functionally immature $CD11c^{+}$DCs expressing low levels of costimulatory CD86 and CD40 molecules in tumor and tumor-draining lymph nodes. Depletion of these DCs in tumor-bearing mice retarded significantly tumor progression. Studies in human gastric and nonsmall lung cancer confirmed that differentiation of DCs was negatively affected by VEGF (Takahashi 2004). Murine studies demonstrated that a population of immature mDCs acquired upon VEGF stimulation a proangiogenic $CD11c^{+}DEC205^{+}$VE-$cadherin^{+}$ phenotype, migrated to perivascular areas of the tumor, and maintained its vasculogenesis (Coukos et al. 2005). Interleukin-10 is responsible for downregulation of costimulatory molecules on DCs, thus cooperating with VEGF in worsening of APC function of DCs. It also blocks DCs' differentiation. The source of IL-10 is tumor itself and TAMs. Similar effects showed exposition of DCs to TGF-β function (reviewed in: Fricke and Gabrilovich 2006). Renal cancer cell lines were shown to produce IL-6 and GM-CSF which

inhibited DCs' differentiation. The blocking effect of IL-6 was also observed in myeloma. Retention of DCs inside tumors and downregulation of their migratory potential are probably mediated by CXCL8 (IL-8) produced by tumors, including hepatocellular, pancreatic, and colon cancers, which act through CXCR1 and -2 receptors on DCs (reviewed in: Fricke and Gabrilovich 2006). Expression of IDO on DCs deprives tryptophan to the T cells and promotes T-cell apoptosis or anergy. The presence of IDO-positive DCs was confirmed in tumor-draining lymph nodes in the cases of melanoma, breast, colon, lung, and pancreatic cancers, and the intensity of such infiltrate was correlated with poor prognosis (Munn et al. 2002). Population of cells which mediated entirely all IDO-dependent suppression in lymph nodes was population of $CD19^+B220^+$ plasmacytoid DCs (Munn et al. 2004). Expression of IDO on DCs is probably upregulated by PGE_2 present in tumor environment. IDO +DCs are capable of inducing $CD4^+CD25^+Foxp3^+$ Tregs. Immature DCs also exert other activating T $CD4^+CD25^+Foxp3^+$ Tregs effects, mediated through TGF-β and IL-10, thus promoting tumor growth (Ghiringhelli et al. 2005; reviewed in: Palucka et al. 2011). Interactions between DCs and Tregs mediated through CTLA-4 could compromise antitumor immunity in an IDO-dependent way. DCs can also modulate the trafficking of Tregs into tumor site and lymph nodes, thanks to CCR4/CXCL22 interactions (reviewed in: Palucka et al. 2011). Tregs were shown to direct back regulatory signals toward DCs, mainly by downregulation of costimulatory molecules on DCs, inhibition of their maturation, and impairment of APC functions by TGF-β and IL-10. Tregs were also reported to induce immunosuppressive molecules B7-H3 and B7-H4 on the surface of DCs (reviewed in: Palucka et al. 2011). Accumulation of ROI in tumor localization creates a constant stress which has profound impact on DCs functions and vulnerability to apoptosis, through modulation of NF-κB and c-Jun N-terminal kinases (JNK) pathways (reviewed in: Fricke and Gabrilovich 2006). Molecule CD200 is a membrane protein belonging to costimulatory molecules, which exerts suppressive effects through binding to CD200 receptor (CD200R). Both CD200 and CD200R are present on the surface of myeloid DCs. It was shown that stimulation of CD200R on DCs created tumor-supporting reactions mediated by Th2 cytokines and increased Tregs activity, while blocking CD200/CD200R interactions with monoclonal anti-CD200 antibodies resulted in a shift toward Th1 activity. Moreover, tumors themselves (including ovarian cancer) are capable of expressing CD200 molecules, thus influencing DCs' function. Myeloid DCs isolated from ovarian tumors also exhibited the expression of programmed cell death-1 ligand 1 (PD-L1, B7-H1). Accumulation of $PD\text{-}1^+B7\text{-}H1^+$ DCs in the tumor was associated with suppression of $TCD4^+$ helper, T $CD3^+CD8^+$ cytotoxic/regulatory cell activity, decreased infiltration of T cells, and expansion of Tregs (Krempski et al. 2011; reviewed in: Palucka et al. 2011). In ovarian cancer, plasmacytoid DCs accumulate in tumor environment, preferentially in ascites, where they are attracted by CXCL12 (Curiel et al. 2004). Similarly to MDCs, ascitic PDCs have immature phenotype. Plasmacytoid DCs promote the generation of immunoregulatory $IL\text{-}10^+$ T $CD8^+$ suppressors, which independently from T $CD4^+CD25^+FoxP3^+$ Tregs downregulate IFN-γ secretion mediated by T effectors and prevent them from proliferation. They also secrete TNF-α and IL-8, thus being

capable of promoting angiogenesis (Curiel et al. 2004). Tumor-associated PDCs were found to have different phenotype compared to ascitic PDCs, and expressed semimature phenotype with higher level of CD86 and CD40 expression, thus being capable of partial activation in tumor localization. Function of tumor-associated PDCs was modulated by tumor-derived TNF-α and TGF-β (Labidi-Galy et al. 2011). The DCs intercellular machinery exposed to tumor-derived regulatory molecules inhibits their differentiation to mature phenotype via induction of STAT3 signaling. Moreover, activation of STAT3 in tumors blocks secretion of proinflammatory factors and enhances DCs immaturity (reviewed in: Palucka et al. 2011).

1.4.5 Inflammation and Cancer Escape

Chronic inflammation may account for about 15% of cancers, due to the fact that inflammation mediators like TNF-α could initiate tumor growth by stimulation of NO synthase and ROI production, both being capable of DNA damage (Balkwill and Mantovani 2001; Li and Karin 2007; Hussain et al. 2003). During progressive tumor growth, chronic inflammation caused by tumor-infiltrating immune cells contributes to cancer progression (Ben-Baruch 2006). Oxidative stress seems to play pivotal role in this process by stimulating inflammatory network based on COX2, iNOS, cytokines, chemokines, and transcription factors. Reactive oxygen intermediates participate in regulation of resistance to apoptosis, angiogenesis, proliferation potential, and metastasis formation (reviewed in: Reuter et al. 2010). Moreover, stromal cells could also contribute to chronic inflammation and initiate or promote tumor growth. Upon senescence, fibroblasts acquire "senescence-associated secretory phenotype" (SASP) characterized by activation and production of proinflammatory cytokines (IL-6, IL-1β), chemokines (IL-8, MCP-1, GRO-1/α), MMPs, adhesion molecules, and integrins (Shan et al. 2009). The senescent stromal fibroblasts were detected in specimens of ovarian tumors in areas adjacent to malignant epithelium. The chronic inflammation and oxidative stress also promote the generation of heat-shock proteins (HSPs), which prevent cells from apoptosis and enhance their survival. Overexpression of HSP90 was found on the several tumors, and correlated to metastatic potential and poor survival. Similarly, the presence of HSP70 was noticed on colon, lung, breast, and pancreatic cancer metastases, and correlated with resistance of cancer cells against apoptosis (reviewed in: Goldstein and Li 2009).

Toll-like receptor polymorphisms in genes encoding TLR6 and TLR10 increased risk of some cancers. Activation of TLR receptors both on macrophages and on the cancer cells enhanced tumor growth by various mechanisms like stimulation of growth-promoting cytokines or protection against apoptosis (Medzhitov 2001). In ovarian cancer, stem-like slow-growing cell population initiates tumor regrowth after surgery or chemotherapy by activation of TLR4-pathway, which regulates proinflammatory phenotype of these cells characterized by high NF-κB, IL-6, IL-8, MCP-1, and GRO-1/α activity (Mor et al. 2011). Therefore, the $TLR4^{+}$

phenotype of ovarian cancer cells was correlated to chemoresistance. Similarly, the expression of TLR9 was connected to high metastatic potential of ovarian tumors.

Tumor necrosis factor-α is one of the proinflammatory cytokines stimulated by TLRs, which promotes tumor survival by stimulation of NF-κB-dependent pathways regulating antiapoptotic molecules, tumor proliferation, neoangiogenesis, and metastatic properties (Elgert et al. 1998). Polymorphisms leading to overproduction of TNF-α were connected with greater risk of cancer, including breast and gastric tumors (Mocellin et al. 2005). Increased TNF-α concentrations were observed in ovarian cancer patients in serum and cyst fluid, as well as in cancer tissues and ascites. Cancer patients were also characterized by overexpression of receptor TNF-R2, which was further correlated with tumor stage and patients prognosis (Dobrzycka et al. 2009). TNF-α expressed on tumor cells orchestrates the paracrine "TNF network" and together with IL-6 and CXCL12 regulates tumor growth (Kulbe et al. 2012). Interactions between tumor-derived IL-6 and TAMs-derived TNF-α enhanced incidence of prostate cancer metastases both to the bones and regional lymph nodes. Moreover, prostate tumors were characterized by increased TNF-α, TNFR1, and TNFR2 levels, which correlated with poor prognosis (Tse et al. 2012).

Interleukin-6 is another proinflammatory cytokine which through activation of intracellular STAT3 pathway regulates cell proliferation, induces epithelial–mesenchymal transition and appearance of cell migratory phenotype, and upregulates resistance to apoptosis and chemoresistance (Hodge et al. 2005). Polymorphisms of the *IL6* gene promoter region could influence the risk of certain tumors (Berger 2004). *In vitro* investigations in ovarian cancer showed that *p53* overexpression could regulate IL-6 secretion (Nash et al. 1999). Interleukin-6 is produced either by tumor cells themselves or by M2-shifted tumor-associated macrophages, and together with IL-1, TNF-α, VEGF, and chemokines produce a cooperative network for promotion of tumor growth (Lane et al. 2011; Kulbe et al. 2012). IL-6 could induce suppressive Th2 phenotype in tumor-infiltrating T cells and M2-type activity in TAMs. *In vitro* studies showed that IL-6 augmented growth of colon carcinoma, which was confirmed *in vivo* by the observation that IL-6 serum levels correlated with the dimensions of the tumor. Increased IL-6 expression was related to advanced stage of disease and decreased survival in colon cancer patients. These effects were mediated through IL-6-mediated promotion of tumor cell proliferation and inhibition of apoptosis through gp130 activation on tumor cells with subsequent signaling through JAKs and STAT3 (Waldner et al. 2012). Women with advanced ovarian cancer had significantly higher IL-6 levels both in the serum and ascites (Clendenen et al. 2011; Nowak et al. 2010a). In these patients, IL-6 was engaged in neoangiogenesis, spread of peritoneal metastases, and ascites production. In several prostate cancer cell lines, IL-6 inhibited apoptosis and enhanced survival by activation of phosphatidylinositol-3-kinase signaling (Culig and Puhr 2012).

Transforming growth factor-β despite its antitumor activity in early tumors might also enhance tumor escape and contribute for tumor-associated inflammation in later stages. Mutations of the TGF-β-receptor, Smad signal transduction pathway genes, and TGF-β-inducible gene-h3 were associated with reduced p53 expression, ovarian cancer risk, and paclitaxel resistance, respectively. On the contrary, some

polymorphisms of *TGF* gene make individuals less prone for development of lung cancer (reviewed in: Jadus et al. 2012). The source of TGF-β could be both tumor cells and M2-type TAMs (Ostrand-Rosenberg 2008). Lung cancers overexpress TGF-β and are characterized by several mutations of TGF-β receptors, which prevent cancer cells from negative autocrine regulation of growth by this cytokine. As a result, high TGF-β concentration produces suppressory environment inside the tumor (reviewed in: Jadus et al. 2012). In advanced tumors, TGF-β is engaged in Th17 cell differentiation, inhibition of DCs maturation, and stimulation of VEGF production, generating the $CD4^+CD25^+Foxp3^+$ Tregs and decreasing activity of NKT, T $CD8^+$, and NK cytotoxic cells. It supports angiogenesis, metastasizing, and epithelial–mesenchymal transition (Moutsopoulos et al. 2008; Yu et al. 2006; Gavalas et al. 2010). In breast cancer, chemotherapy-induced TGF-β signaling enhances tumor recurrence through IL-8-dependent expansion of CSCs, while TGF-β pathway inhibitors prevent the development of drug-resistant CSCs. TGF-β signaling induces mTOR complex 2 in cancer cells and regulates epithelial–mesenchymal transition (Moutsopoulos et al. 2008; Yu et al. 2006).

Interleukin-10, similarly to TGF-β, exerts both antitumor and protumor activity, which seems to be dependent on the tumor type and advance of the disease. IL-10 was shown to be secreted directly by tumor cells, as well as by immunoregulatory Tr1/Th3, $CD4^+CD25^+Foxp3^+$ Tregs, TAMs, and MDSCs. In established tumors, IL-10 enhances the intratumor and peripheral blood immunosuppressive phenotype by stimulation of M2-type TAMs and Th2-type lymphocytes (Rabinovich et al. 2010; Seo et al. 2002; Moutsopoulos et al. 2008; Yu et al. 2006). Autocrine activation of the STAT3 pathway by IL-10 in tumor cells upregulates expression of Bcl-2 and HLA-G, thus protecting cancer cells from host effectors and apoptosis (Urosevic and Dummer 2008). In ovarian cancer patients, IL-10 concentrations were increased in peritoneal fluid and serum compared to benign ovarian disease (Nowak et al. 2010). Moreover, the expression of IL-10 was found to correlate with tumor aggressiveness, the presence of metastases, and shorter progression-free survival (Matte et al. 2012). High levels of IL-10 in TAMs significantly correlated with stage, tumor size, lymph node metastasis, lymphovascular invasion, or histologic poor differentiation in nonsmall cell lung cancer. In melanoma patients, IL-10 mRNA expression increased progressively from preinvasive, through primary invasive to metastatic tumors, and correlated with vertical growth phase as well as metastatic competence (Itakura et al. 2011).

Cyclooxygenase-prostaglandin E_2 inflammatory pathway is important for tumor development, as revealed by studies showing antitumor effects of selective COX2 inhibitors in colorectal cancer (Wang and DuBois 2006). Activity of *cox2* gene was proved to participate in ovarian carcinogenesis both in sporadic and in BRCA 1/2-conditioned cancers. Upregulation of COX2-PGE_2 in tumor cells and TAMs results from hypoxia and HIF-1α, and influences several regulatory and signaling pathways including Ras/mitogen-activated protein kinases (MAPK), phosphatidylinositol 3-kinase/phosphatase (PI3K)/AKT, and NF-κB-mediated pathway (Wang and DuBois 2006). COX2 overexpression stimulates VEGF and neoangiogenesis, and its raised levels predict poor survival in some cancers (Zhang and Sun 2002). In

murine model, COX inhibitors administered together with taxol decreased the expression of VEGF and reduced microvessel density (MVD) of transplanted ovarian tumors. Overexpression of COX2 in ovarian cancer also correlated with resistance to platinum-based chemotherapy. COX2, microsomal prostaglandin E synthase-1 (mPGES-1), and prostaglandin receptor EP1 were positive not only in tumor epithelial cells, but also in the tumor stroma, indicating that CAFs participate in the COX/PGE_2 signaling. Lung cancers also overexpress COX2 and produce several prostanoids and leukotrienes. The presence of COX2 overexpression seems to be the key factor in promotion of lung cancer growth, as the pharmacologic inhibition of COX2 reduced tumor growth in lung cancer murine model. COX2 was capable to modulate MDSCs' activity through PGE_2-mediated ARG-1 expression and to enhance expansion of Tregs also by PGE_2 (reviewed in: Srivastava et al. 2012). PGE_2 inhibits DCs' maturation and migration toward regional lymph nodes, upregulates IL-4 and IL-10 cytokines, and finally increases tumor migratory and metastatic potential (Wang and DuBois 2006; Bennaceur et al. 2009). Squamous, adenocarcinoma, and small cell lung cancers are able to produce prostaglandin E_2 and express a variety of prostaglandin receptors. PGE_2 functions as stimulator of lung cancer growth by augmenting angiogenesis and proliferation, and simultaneously inhibits T and NK effector cells (reviewed in: Jadus et al. 2012). Peroxisome proliferator-activated receptor-γ (PPARγ) is an inhibitor of COX-dependent inflammatory reaction, and in mouse studies produced decrease of PGE_2 levels, reduction of MVD, enhanced tumor apoptosis, and improved mice survival.

Proinflammatory cytokine IL-23 also documents the relationship between the cancer and inflammation. In ovarian cancer, particularly high level of expression of genes regulating proinflammatory pathway including IL-23 was detected. Moreover, *IL-23* receptor gene polymorphism was shown to correlate with advancement of tumors. Upon stimulation by tumor-derived IL-23, Th17 cells release IL-17 and other inflammatory mediators like IL-1, IL-8, TNF-α, and PGE_2 which produce protumor inflammatory environment. The increased expression of both IL-23 and IL-17 was observed in many malignant tumors and correlated with angiogenesis, expression of MMPs, and decrease of cytotoxic antitumor immune response (Langowski et al. 2007; Whiteside 2010).

Interleukin-18 is a proinflammatory cytokine which activates immune CTL and NK cells and induces IFN-γ, thus is capable to exert antitumor effects. However, IL-18 was also found to potentiate tumor growth (reviewed in: Park et al. 2007). The expression of IL-18 was demonstrated on melanoma, squamous skin, breast, and gastric cancers, and was connected with the presence of distant metastases in breast and gastric cancers. *In vitro* studies showed that transfection of poorly metastatic lung cancer cells with IL-18 construct enhanced their invasion ability and downregulated E-cadherin, thus increasing metastasis potential (Jiang et al. 2003). In murine melanoma model, the prometastatic IL-18 action was mediated by upregulation of vascular cell adhesion molecule 1 (VCAM-1). Also the proangiogenic properties of IL-18 were noticed in gastric cancer, where IL-18-dependent stimulation of thrombospondin-1 was discovered. Moreover, IL-18

induced Fas ligand expression on melanoma cells and makes them less susceptible for effector destruction (reviewed in: Park et al. 2007).

Interleukin-8 (also CXCL8) is a chemokine secreted by macrophages, neutrophils, endothelial, and tumor cells, mediating its biological effects through binding to CXCR1 and CXCR2 receptors present on both tumor and endothelial cells (Walz et al. 1987; Murdoch et al. 1999; Xu and Fidler 2000). Hypoxia and oxidative stresses are strong inducers of IL-8 expression on cells of several malignancies, including ovarian cancer, via *Ras* gene overexpression and activation of PI3K/AKT and p38 MAPK signaling (Xu et al. 2004). Some *IL-8* gene polymorphisms are correlated with the overall risk of developing the intestinal type of gastric cancer (Xue et al. 2012). Increased IL-8 was found in ascites and serum of ovarian cancer patients, while IL-8 overexpression was observed on tumor cells, both correlated with advancement, vascularity of tumors, and short patient's survival (Uslu et al. 2005, Merritt et al. 2008). IL-8 is engaged in blocking of TRAIL-induced cancer cells apoptosis and in recruiting certain immune cells into peritoneum, where they contribute to tumor spread and formation of ascites (Wang et al. 2006, Abdollahi et al. 2003). It was shown that chemoresistant ovarian cancers were characterized by increased expression of IL-8 (Duan et al. 1999). IL-8 and CXCR1 were found to be overexpressed in pancreatic cancer, and *in vivo* studies showed that tumors from patients who had higher IL-8 levels grew faster (Chen et al. 2012). *In vitro* studies of gastric cancer revealed that IL-8 increased NF-κB and AKT signaling and adhesion molecules intercellular adhesion molecule (ICAM-1) and VCAM-1 expression in cancer cells, thus increasing their migration, adhesion, and invasion (Kuai et al. 2012). Similarly, IL-8-transfected colon cancer cell lines demonstrated increased migration and proliferation *in vitro*, whereas *in vivo* xenografted IL-8-expressing colon tumors indicated faster growth and enhanced microvessel density (Ning et al. 2011). Overexpression of CXCR2 receptor inhibited cancer apoptosis, upregulated VEGF on tumor cells, and was an indicator of poor prognosis (Yang et al. 2010).

Hedgehog (Hh) signaling pathway plays important role in human development. The expression of Hh ligands and the intensity of Hh signaling are upregulated by hypoxia and inflammation (Bijlsma et al. 2009, Pratap et al. 2010). Classical activation way requires binding of one of Hh ligands (Sonic—SHH, Indian—IHH or Desert—DHH) to the membrane-bound receptor Patched (PTCH). The Hh-PTCH complex influences the Smoothed (SMO) factor which activates the glioma-associated oncogene homolog (GLI) transcription factors that upregulate target genes (reviewed in: Harris et al. 2011). During embryonic development, Hh signaling promotes cell proliferation, angiogenesis, EMT, and stem cell regrowth, all under hypoxic conditions; thus, situation according to the Hh function resembles in some circumstances that inside solid tumors. Inhibition of Hh signaling was found to decrease the proliferation of cancer cells (Berman et al. 2002). The Hh-GLI-mediated increase of proliferation was observed in melanoma cells (Stecca et al. 2007). Target genes responding to Hh-GLI regulation include proliferation activators including cyclins, IGF-BP6, and osteopontin. Moreover, Hh-GLI pathway upregulates the expression of Bcl-2 antiapoptotic molecule (in brain, gastric, and pancreatic cancers) and regulates stability of p53 (in breast cancer) (Yoon et al.

2002, Wang et al. 2010, Das et al. 2009, Han et al. 2009, Abe et al. 2008). In ovarian and endometrial cancer, Hh signaling downregulates the p21 and p27 inhibitors of cell-cycle progression, and correlates with advancement of the tumors (Feng et al. 2007, Liao et al. 2009). The Hh-GLI pathway is also engaged in angiogenesis via upregulation of VEGF, and enhances invasiveness and migration in several tumors including skin, breast, ovarian, pancreatic, prostate cancers, and melanoma (reviewed in: Harris et al. 2011). It also represses E-cadherin expression, enhances MMPs, and activates stromal fibroblasts, thus inducing EMT (Li et al. 2007b; Yoo et al. 2008; Dunér et al. 2011). One of the most important functions of Hh signaling is the maintenance of the CSCs, with slow-proliferating, self-renewing population of cells being the reservoir for tumor regrowth (Li et al. 2007). The stimulatory effect of Hh on viability of CSCs was observer in variety of tumors including breast, brain, ovarian, and colon cancers (reviewed in: Harris et al. 2011).

1.4.6 Resistance to Apoptosis and Tumor "Counter Attack"

Apoptosis describes the highly selective process, occurring both in physiological and pathological circumstances, by which cells upon receiving certain activating stimuli enter the course toward a programmed death (Kerr and Harmon 1991). Resistance to apoptosis or its reduced efficacy has been repeatedly reported as one of the escape mechanisms observed in the cancer development. The background for these phenomena could originate from disturbances of merely all steps of apoptotic pathway inside tumors, including disrupted pro- and anti-apoptotic signaling, impaired caspase activity, and defective death receptor function (reviewed in: Wong 2011). Some reports suggest that polymorphic variations in genes regulating apoptosis could interfere with the risk of cancer. An association with several cancer types and *TNFalpha* gene or *FAS* promoter region polymorphisms has been found (Balkwill 2002, Lai et al. 2003, Sun et al. 2004). On the contrary, presence of certain *DR4* and *CASP8* polymorphisms could have a protective effect against bladder and breast cancers, respectively (Hazra et al. 2003, MacPherson et al. 2004). Downregulation of apoptosis mechanisms observed in tumor cells could augment tumorigenesis by influencing proliferative capabilities and drug resistance of the cancer. The next problems are resistance of tumors to T-cell-dependent cytotoxicity and apoptosis, and a tumor cell "counter attack" against host immune effector cells using apoptotic pathway.

Apoptosis-regulatory proteins that have been extensively studied in solid cancers belong to Bcl-2 family proteins or inhibitors of apoptosis. The Bcl-2 family of proteins is engaged in intrinsic pathway of apoptosis and acts in mitochondria-dependent way (Gross et al. 1999). The mutations of proapoptotic proteins and overexpression of antiapoptotic proteins were observed in the cases of solid tumors. In transgenic mice having an enforced expression of Bcl-2 protein, an increased risk for cancer incidence occurred; however, it was rather low (about 10%) and tumors developed in advanced age (Cory et al. 2003). Hence, although Bcl-2 mutation is

causally connected with origin of cancer, it does not seem to be the only sufficient condition for malignant transformation. Bcl-2 rather promotes neoplastic transformation and, by prolonging the lifespan of the cells, allows them to accumulate additional oncogenic mutations (Zhivotovsky and Orrenius 2006). Observation that double transgenic mice, overexpressing products of both *bcl-2* and *c-myc* genes, show accelerated appearance of breast cancer seems to confirm that notion (Jager et al. 1997). Overexpression of Bcl-2 protein was shown in prostate and breast cancers, and led to inhibition of TRAIL-mediated apoptosis (Raffo et al. 1995, Fulda et al. 2000). Bcl-2 is also highly expressed in small cell lung cancer and to a lesser extent in squamous lung cancers (reviewed in: Jadus et al. 2012). Some other members of the Bcl-2 family could also participate in tumorigenesis. Bcl-w protein was overexpressed in both colorectal and gastric adenocarcinomas, and it was shown to suppress cell death by blocking JNK activation pathway (O'Reilly et al. 2001, Lee et al. 2003). Colorectal cancers characterized by microsatellite instability demonstrated the presence of mutations in the *bax* gene resulting in impaired function of proapoptotic Bax protein (Miquel et al. 2005). The stable tumor cell lines overexpressing Bcl-xL protein were found to be apoptosis- and drug-resistant (Minn et al. 1995).

Inhibitor of apoptosis proteins (IAPs) are endogenous inhibitors of caspases. Amplification of chromosomal regions which encompass the IAPs-coding sequences was observed in various tumors including esophageal squamous cell carcinoma (Zhivotovsky and Orrenius 2006). The upregulation of IAPs family members' expression was documented in various cancers, including pancreatic cancer and glioma, and was responsible for chemoresistance (Lopes et al. 2007, Chen et al. 1999b). Overexpression of survivin, another extensively studied member of IAPs family, was demonstrated in nonsmall cell lung carcinoma (Krepela et al. 2009). In neuroblastoma, expression of survivin was correlated with more aggressive and unfavorable disease (Adida et al. 1998).

Another example of apoptosis-regulatory protein that has been studied is p53 suppressor protein, due to its multidirectional function frequently called the "guardian of the genome" (Wong 2011, Lane 1992). The p53 protein, found to be downregulated in numerous cancers, functions as a regulator of some target genes involved in apoptosis resistance and increased proliferation activity of melanoma (Avery-Kiejda et al. 2011). It was also shown that silencing of p53 mutants in cancer cell lines resulted in reduced cellular growth due to increased apoptosis (Vikhanskaya et al. 2007). Point mutations of p53 occurring frequently in lung cancers caused upregulation of Bcl-2 with concomitant Bax hypoexpression (reviewed in: Jadus et al. 2012).

Reduced caspase activity is another mechanism of cancer apoptosis resistance. Caspases form the system of cytoplasmic enzymes engaged in inflammatory cytokine processing and apoptosis. Mutations of the *caspase-8* gene, including modification of stop codon, missense mutation at the codon 96, and the deletion of the leucine 62, were found in head and neck cancer, neuroblastoma, and vulvar squamous cancer, respectively (Mandruzzato et al. 1997, Takita et al. 2001, Liu et al. 2002). All of them prevented the proper activation of the caspase cascade. Similarly,

silencing mutations in caspase-9 gene were associated with development of neuroblastoma and small cell lung cancer (Catchpoole and Lock 2001, reviewed in: Jadus et al. 2012). Loss of caspase-1 mRNA was observed in gastric cancer and metastatic melanoma, and in both tumors correlated with clinical stage and bad prognosis (Jee et al. 2005, Mouawad et al. 2002). Both downregulation of caspase activity and their decreased concentrations were described in various tumors, including colorectal, ovarian, breast, and cervical cancers, the fact that was correlated with poor clinical outcome (Shen et al. 2010, Devarajan et al. 2002). A deficiency of caspase-8 was described in small cell lung cancer and neuroblastoma (Joseph et al. 1999, Fulda et al. 2001). And conversely, high levels of caspase-3 inside the tumors cells correlated with low malignancy and good outcome in pancreatic and lung cancers (Volm and Koomagi 2000; Koomagi and Volm 2000). However, dysregulation of apoptosis observed in some studies seems to be much more complex and does not allow for simple conclusions. Expression of caspase-3 and -7 did not correlate with clinicopathological features of breast cancer (Grigoriev et al. 2002), and active caspase-6 concentrations were increased in progressive melanoma and its metastases compared to nonmalignant naevi (Woenckhaus et al. 2003). Therefore, despite the fact that disturbances of apoptosis regulation in various tumors are obvious, there is still no certainty regarding the problem whether these disturbances are primary or secondary events in cancer (Zhivotovsky and Orrenius 2006).

The death receptors Fas (CD95) and TRAILR1 and -R2 are the members of the TNF receptor superfamily characterized by the presence of intracellular death domain (DD), and together with their ligands, FasL and TRAIL play important role in the regulation of extrinsic apoptosis pathway. Tumors are able to inhibit the death receptor signaling at several steps. The spectrum of possible disturbances covers the downregulation or impairment of receptor function and the reduced level of the death signals (Wong 2011). Loss of Fas was attributed to mutations in *ras* and *TP53* genes (Peli et al. 1999, Volkmann et al. 2001). Tumor-associated mutations could also deregulate the function of Fas and TRAIL receptors. Missense mutations and loss of *Fas* gene were identified in myeloma and melanoma (reviewed in: Khong and Restifo 2002). Deletions and mutations of TRAILR1 and -R2 receptors were detected in many tumors, including nonsmall cell lung cancer (reviewed in: Igney and Krammer 2002). Lack of cytoplasmic signaling domains of Fas and TRAILR1 and -R2 was found in many tumors, including myeloma, gastric, and breast cancers (reviewed in: Töpfer et al. 2011). Inactivating mutations of downstream Fas signaling molecules like FADD and caspase-10 were found in nonsmall cell lung cancer (Shin et al. 2002). The low expression of Fas and both FasL and TRAIL was documented in neuroblastoma and precancerous cervical lesions, respectively (Fulda et al. 1998, Reesink-Peters et al. 2005). High levels of antiapoptotic regulator FLICE inhibitory protein (c-FLIP) were demonstrated to correlate with TRAIL-mediated apoptosis in melanoma cells (Griffith et al. 1998). Overexpression of c-FLIP was confirmed in several tumors in mice and humans and, in some of them, was correlated to bad prognosis (reviewed in: Töpfer et al. 2011; Igney and Krammer 2002).

Activation of T cells during immune response is a self-limiting phenomenon, as activated T cells upregulate Fas death receptor and enter activation-induced cell death (AICD). Some tumors, like melanoma, lung, pancreatic, gastric, colon, and breast cancers, might accelerate AICD and escape from immune recognition and destruction, by overexpression of FasL and elimination of T effectors in FasL-dependent pathway (reviewed in: Töpfer et al. 2011, Kim et al. 2004). Expression of FasL on their surface is either constitutive or induced by chemotherapy (reviewed in: Igney and Krammer 2002). A significant reduction in TILs and apoptosis of Fas-positive TILs was observed in esophageal cancer and metastatic gastric carcinoma, respectively. Similar correlation was found in head and neck tumors and ovarian cancer. The expression of FasL and TILs apoptosis was more evident in metastatic colon cancer and in breast cancer lymph node metastases. High FasL/Fas ratio was a bad prognostic sign among patients with ovarian and hepatocellular cancers (reviewed in: Kim et al. 2004). The meaning of FasL for tumor escape is sustained by observation that downregulation of FasL expression in colon cancer cells significantly reduced tumor growth in syngeneic mice and stimulated T-cell antitumor response (Ryan et al. 2005). Moreover, soluble FasL (sFasL), which is produced by cleavage of membrane FasL by tumor metalloproteinases, as well as microvesicles containing FasL produced and released by melanoma, could kill effector immune cells and cause systemic immunosuppression (Andreola et al. 2002, reviewed in: Kim et al. 2004). Significantly increased number of $CD3^+Fas^+$ apoptotic T cells was found in blood of patients with metastatic melanoma and head and neck cancers. Furthermore, T $CD8^+$ cells more frequently entered apoptosis than T $CD4^+$ cells, suggesting that T $CD8^+$ cells are more sensitive to apoptosis (Dworacki et al. 2001, Hoffmann et al. 2002). These mechanisms were called FasL "counter attack" (Hahne et al. 1996). It is directed against tumor-infiltrating and by-standing T lymphocytes, as upon tumor recognition T cells express substantial levels of FasL which induces "suicidal" and "fratricidal" T-cell death (reviewed in: Rabinovich et al. 2007, Khong and Restifo 2002). Moreover, human metastatic melanoma cells are capable to engulf and ingest T lymphocytes in a process called "tumor cannibalism" (Lugini et al. 2006). However, the function of FasL can also accelerate the rejection of tumor by induction of proinflammatory and antitumor effects mediated *in vivo* by activated neutrophils (Arai et al. 1997). In addition, screening of the melanoma cell lines by RT-PCR and functional assays did not reveal expression of functional FasL (Chappell et al. 1999). To summarize these conflicting results, it was hypothesized that the local levels of FasL may determine the course of the events, with high FasL levels provoking neutrophil infiltration, and lower levels being capable of antitumor T responses elimination. Activation of neutrophils might depend on the form of FasL (only membrane-bound FasL is an activator) and/or on the macrophages and DCs which upon FasL stimulation produce IL-1β and other proinflammatory proteins and chemoattractants (Igney and Krammer 2002). The extension of FasL/Fas signaling could be genetically determined, as different tumors are characterized by either frequent or rare Fas mutations, and p53 mutation abundantly met in various tumors can downregulate Fas expression (reviewed in: Kim et al. 2004). The effects of FasL/Fas signaling might also

depend on the local environment, which through the action of some immunoregulatory molecules may create an appropriate condition to tumor escape. Upregulation of FasL on tumor cells resulted from proinflammatory cytokines TGF-β, IL-10, prostaglandins, and reactive oxygen species (reviewed in: Rabinovich et al. 2007, Kim et al. 2004).

Other molecules including RANTES and receptor-binding cancer antigen expressed on SiSo cells (RCAS1) could augment FasL "counter attack" by inducing cycle arrest and apoptosis of antitumor-activated T cells (reviewed in: Rabinovich et al. 2007, Khong and Restifo 2002). Tumor cells also showed ability to use a transmembrane or soluble decoy receptors with nonfunctional or absent death domain to avoid T-cell-mediated apoptosis. Decoy receptors, like soluble Fas (sFas) or various TRAIL receptors (−R3, −R4), have been described in tumors (reviewed in: Töpfer et al. 2011). Increased serum level of sFas was detected in various tumors, and correlated with poor outcome in melanoma patients (reviewed in: Igney and Krammer 2002). T cells can also eliminate target cells by the perforin/granzyme pathway. It was demonstrated that tumors are resistant to perforin/granzyme-dependent killing by cytotoxic T cells, caused by the expression of granzyme B inhibiting serine protease inhibitor PI-9/SPI-6 present on the cells of melanoma, cervical, and breast cancers, and correlated with a poor patients outcome (Medema et al. 2001, van Houdt et al. 2005). Another immunological mechanism that contributes to cancer "counter attack" against cytotoxic T cells involves the interactions between PD-1 and its ligand PD-L1, also called B7-H1. Different tumors including ovarian, colon, lung, and breast cancers indicate the expression of PD-L1, similar to tumor-infiltrating myeloid cells in nonsmall cell lung cancer (Jadus et al. 2012). Binding of PD-1 on T cells to its ligand on cancer cells resulted in inhibition of T-cell activation via induction of FasL and IL-10. Moreover, blocking of PD-L1 reduced T-cell apoptosis in tumor models (Rabinovich et al. 2007, Keir et al. 2008, Chemnitz et al. 2007). Overexpression of PD-L1 on ovarian cancer epithelial cells is a mechanism of possible importance for intraepithelial T $CD8^+$ depletion and deactivation (Hamanishi et al. 2007). Lung tumors possessing high expression of PD-L1 showed less TILs compared to B7-H1-negative tumors (reviewed in: Jadus et al. 2012). The precise mechanism of PD-1/PD-L1 interactions is probably based on upregulated expression of the activator protein-1 (AP-1) subunit c-Fos in TILs. Immunosuppressive effect of c-Fos was mediated through induced expression of PD-1 via connection of c-Fos to the AP-1-binding site in PD-1 encoding gene. Knocking-out mutation of this binding site abrogated PD-1 induction and augmented T effector immunity (Xiao et al. 2012). Tumor cells subjected to apoptosis generate apoptotic bodies, a structure distinct from microvesicles and exosomes, which are formed from randomly blobbing cellular membrane vesicles having a couple of micrometers in diameter. They contain fragmented nuclei and organelles, and are able to transfer oncogenes into target cells and to suppress cytotoxic antitumor T $CD8^+$ lymphocytes (reviewed in: D'Souza-Schorey and Clancy 2012).

1.4.7 The Role of Tumor Stroma in Immune Escape

Solid tumors are composed not only of neoplastic cells, but also of stroma containing fibroblasts, extracellular matrix, endothelial cells, and tumor-infiltrating immune cells. One of the most important population of cells which are residents in tumor stroma are CAFs. These cells met with growing interest, due to their capabilities to initiate and promote tumor growth (reviewed in: Östman and Augsten 2009). The population of CAFs gathers distinct subpopulations of fibroblasts; however, their precise functions and differences between them still await investigation. Another interesting question is origin of CAFs. Most of them are modified local fibroblasts, but some additional sources of CAFs have been identified, which vary according to the tumor type. Some cells originate from mesenchymal stem cells, and some are a result of EMT mechanism (reviewed in: Franco et al. 2010). The meaning of CAFs for tumor development is highlighted by the observation that for effective carcinogenesis the presence of cancer cells is not enough, and without a cooperation with surrounding tissues, cancer cells cannot form an aggressive tumor. The interaction between the fibroblasts and ECM in cancer reminds processes of tissue repair, however, disturbed during carcinogenesis (reviewed in: Franco et al. 2010). CAFs produce growth factors exerting tumor-promoting activity, like epidermal growth factor (EGF), FGF, TGF-β, platelet-derived growth factor (PDGF), or IGF (Kalluri and Zeisberg 2006, Östman and Heldin 2007). The population of CAFs also showed expression of chemokines CCL5, CXCL12, and CXCL14, which are responsible for tumor metastatic potential (Karnoub et al. 2007), increased angiogenesis (Orimo et al. 2005), and influx of macrophages into the tumor (Augsten et al. 2009). Previous studies showed that CAFs are an alternative source of VEGF-A capable of compensating the lack of tumor-derived VEGF-A (reviewed in: Ferrara 2010, Kammertoens et al. 2005). These factors act in paracrine manner together with signaling from ECM components and integrins. CAFs-derived TGF-β modulates the growth and the oncogenic potential of adjacent epithelial cells, and promotes their resistance to apoptosis by upregulation of NF-κB transcription factor (reviewed in: Franco et al. 2010). Elevated TGF-β in tumor stroma activates CXCR4 expression in epithelial cells, making them unresponsive to growth-inhibitory signals. Expression of CXCR4 in prostate cancer is a bad prognostic sign (Akashi et al. 2008). IGF-1 expressed by prostate tumor stroma stimulates proliferation of epithelial cells by upregulation of MAPK, AKT, and cyclin D1. In murine model, overexpression of IGF-1 by CAFs promotes malignant transformation of epithelial cells and increases metastatic potential which could be abrogated by blockade of IGF-1 receptor or MAPK. Activation of IGF-1 interferes with TGF-β intercellular Smad pathway and blocks apoptosis of epithelial cancer cells (Saikali et al. 2008). The cooperation between endothelial cells and CAFs could influence carcinogenesis in prostate cancer. Genetic instability of stromal fibroblasts reported in the patients contributes to malignant transformation of epithelial cells (Hayward et al. 2001, Macintosh et al. 1998). Similarly, the murine studies of breast cancer indicated that implantation of tumor cells together with fibroblasts not responding to

TGF-β into laboratory animals augmented growth and metastases of implanted cancer (Cheng et al. 2005). The presence of fibroblasts was not an indispensable condition for tumor growth stimulation *in vitro*, as supernatants from fibroblast culture were also activators of cancer progression, due to the presence of chemokines CXCL12 and CXCL14. Alternations of expression of many genes regulating fibroblast function were noted in breast cancer (reviewed in: Franco et al. 2010). Pancreatic adenocarcinoma, which is one of the most lethal human malignancies, is characterized by intense stromal reaction. CAFs in pancreatic cancer produce ECM proteins, growth factors, and proinflammatory cytokines (Aoki et al. 2006).

During some physiologic processes, like embryonic development and wound repair, there is a temporal need for epithelial cells to escape from the rules governing the tissue structure and adopt a mesenchymal phenotype which enables them to migrate. This is called epithelial-to-mesenchymal transition (EMT) and occurs also in pathological conditions during cancer development and progression. The EMT is an active process during which epithelial cells loose intercellular connections and acquire migratory capacities (Bates and Mercurio 2005). Cell adhesion molecule epithelial E-cadherin belongs to the key negative regulators of EMT, which are responsible for adherens junctions and epithelial integrity. Repression of E-cadherin is regulated by transcription factors called SNAIL, TWIST, ZEB, and SLUG. Loss of E-cadherin functions is a typical phenomenon met in human cancers, thus leading to EMT, decreased adhesion, and increased metastasizing capacity (reviewed in: Bates and Mercurio 2005; Srivastava et al. 2012). Disturbed function of E-cadherin could depend on genetic mutations in its gene; however, most reasons cause inactivation of E-cadherin by promoter methylation and transcriptional repression (Becker et al. 1994; Hirohashi 1998). The initiating signal for EMT is delivered by both tumor- and stroma-derived TGF-β which cooperates with activated Ras pathway (Bhowmick et al. 2001; Fujimoto et al. 2001). EMT accelerates significantly upon TNF-α costimulation with TGF-β (Bates and Mercurio 2005). Following the changes of E-cadherin functions, the alterations in expression of integrin αvβ6 receptor for fibronectin and tenascin occur. The inflammation and tissue repair mechanisms are both the stimulators of this change (reviewed in: Bates and Mercurio 2005). Upregulation of αvβ6 integrin enhances the capability of colon cancer epithelial cells to migrate into the extracellular matrix and to metastasize into liver, and reversely stimulates TGF-β secretion, thus providing the self-perpetuating loop (Busk et al. 1992; Kemperman et al. 1995). As a result of EMT, a single cancer cell migrates in the absence of any intercellular contact, and their survival depends on the autocrine VEGF/Flt1 interactions (Bates et al. 2003). Snail transcription factor expression was confirmed in nonsmall cell lung cancer and melanoma, and correlated with shorter survival and predisposition to metastases, respectively (Yanagawa et al. 2009; Kudo-Saito et al. 2009). Murine studies indicated that snail expression affects the function of MDSCs, as snail-knockout mice were characterized by reduced number and arginase activity (reviewed in: Srivastava et al. 2012).

1.4.8 *Microvesicles and Exosomes—Mediators of Tumor Escape*

Microvesicles are small membrane-enclosed structures shed from the variety of cells, including cancer, which are present in both physiological and pathological conditions in body fluids, like blood, urine, or ascites. Tumor-derived microvesicles (alternatively called oncosomes or ectosomes) are uniquely generated by tumor cells. Microvesicles are a unique population of structures which are distinct from exosomes. Microvesicles originate from an outward budding and fission of the cellular membrane, and may have irregular shape and dimensions ranging from 200 nm to 1 μm (Muralidharan-Chari et al. 2010). Shedding of microvesicles is not just a passive process, as it occurs in specific places of the cell surface, needs exposure of phosphatidylserine, and requires energy input, RNA synthesis, and protein translation (Muralidharan-Chari 2010; Dainiak and Sorba 1991). However, compared to normal cells, tumor cells could shed microvesicles from entire surface, especially from the invading cellular edges (Giusti et al. 2013). The function and contents of microvesicles depend on the cell type which they originate from (Piccin et al. 2007). Tumor-shed microvesicles contain cytokines, miRNA, mRNA, FasL, chemokine receptors, tissue factor, EGFR, Her-2, metalloproteinases, or other molecules (reviewed in: Muralidharan-Chari 2010). Cellular proteins are selectively incorporated into microvesicles in ARF6-regulated endosome recycling, which activation has been linked to acquisition of invasive potential by the tumor (reviewed in: D'Souza-Schorey and Clancy 2012). The interaction with the cells occurs via microvesicle fusion with the target cell or their endocytosis. Microvesicles are released into the body fluids or extracellular milieu, where they play a regulatory role for ECM degradation and invasion, angiogenesis, metastases, and immune escape of the tumor (Valenti et al. 2007). It was demonstrated in mouse model that microvesicles shed from highly metastatic melanoma cells were able to change the phenotype of weakly metastatic melanoma cell line into aggressive phenotype capable of metastasizing (Poste and Nicolson 1980). Similarly, the oncogenic receptor EGFRvIII found on the aggressive gliomas was transferred to a nonaggressive population of tumors (Al-Nedawi et al. 2008). Moreover, the number of microvesicles was shown to correlate with invasiveness of tumor *in vitro* and *in vivo* (Ginestra et al. 1999). Similarly, early stages of ovarian cancer were characterized by lower number of microvesicles in malignant ascites compared to advanced disease (Graves et al. 2004). Microvesicles containing mRNA, miRNA, or fragments of genomic DNA could influence the transcriptome of the target cells and augment tumor invasiveness (reviewed in: D'Souza-Schorey and Clancy 2012). Tumor-derived microvesicles stimulate endothelial cells and stromal fibroblasts to promote neoangiogenesis and invasion. Cancer cell lines were able to produce microvesicles containing VEGF, MMPs, and miRNA which stimulated motility, invasiveness, and tubule formation by endothelial cells. Upon stimulation, the endothelial cells produced their own microvesicles with encapsulated MMPs, VEGF, and esfingomielin which in autocrine manner further promoted endothelial

invasion to the stroma. Those processes were stimulated by hypoxic conditions (reviewed in: Muralidharan-Chari et al. 2010). Microvesicles released by prostate cancer and lung cancer cell lines were shown to chemoattract and activate stromal fibroblasts, and by MMPs increased their motility and resistance to apoptosis. In turn, stimulated fibroblasts were capable of shedding microvesicles facilitating tumor invasiveness and migration (Castellana et al. 2009; Wysoczynski and Ratajczak 2009). Fusion of microvesicles produced by human melanoma and colon cancer cells with monocytes inhibited their differentiation and switched them to immunosuppressive activity. On contact with tumor vesicles, monocytes acquired $CD14^{+}HLA\text{-}DR^{-}$ phenotype, indicated lack of costimulatory molecules upregulation, and started to secrete TGF-β (Valenti et al. 2006). Fas-containing cancer-derived microvesicles induced apoptosis of T cells and abrogated their killing abilities (Wysoczynski and Ratajczak 2009). Tumor cells can escape effector immune cells-mediated apoptosis by preventing the intracellular accumulation of caspase-3, and abrogating of microvesicles production was shown to increase of caspase-3 and apoptosis of tumor cells (reviewed in: Giusti et al. 2013). Presence of MMPs and other proteases inside tumor-derived microvesicles was correlated both *in vivo* and *in vitro* with acquisition of invasive capacity in ovarian and breast cancer, respectively. Activity of proteases within vesicles was augmented in hypoxic environment and played probable role in upregulation of tumor-metastasizing capacity (reviewed in: Muralidharan-Chari et al. 2010). Association between the presence of tissue factor (TF)-containing microvesicles shed from the tumor and increased risk of thromboembolism suggests their role in hypercoagulative state observed in cancer patients (Zwicker et al. 2009). And finally, microvesicles could participate in tumor chemoresistance, as tumors treated with doxorubicin and cisplatin demonstrated shedding of microvesicles containing accumulated, high-concentrated drugs (Shedden et al. 2003; Safaei et al. 2005).

Exosomes originate from reverse budding of the membrane of intracellular multivesicular bodies (MVB) and are released upon fusion with cellular membrane to extracellular fluid or circulation. They form round- or oval-shaped structures and have 30–100 nm of diameter (reviewed in: Zhang et al. 2012). Release of exosomes is regulated by calcium ionophores, phorbol esters, and inositol 3-kinase inhibitors, as well as indirectly by p53 (Clayton et al. 2001, Yu et al. 2009). Exosomes may contain numerous proteins, mRNA, miRNA, lipids, and other active molecules, and influence the cells locally in autocrine and paracrine manner, as well as can regulate the function of distant cells. Exosomes may impact various cellular responses and are engaged especially in regulation of inflammatory processes (reviewed in: D'Souza-Schorey and Clancy 2012). The presence of signal molecules on the exosomes' surface directs them to the target cells and provides their endocytosis or phagocytosis (Thery et al. 2002). Endocytosis of exosomes is energy-consuming process which may occur in clathrin-dependent way and additional endocytosis mechanisms, and which needs both proteins included in exosome and proteins of target cell (Escrevente et al. 2011). Exosomes are produced by various cancers, including melanoma, breast, prostate, and colorectal cancers, and contain specific proteins dependent on the cancer type. The presence of exosomes was confirmed in

vascular circulation, body fluids, and malignant ascites (reviewed in: Zhang et al. 2012). Studies performed on the mouse model of cancer demonstrated that transplantable breast tumors were capable to accelerate growth by releasing exosomes which decreased the number and cytotoxic activity of NK cells. The *in vitro* effects of exosomes originated from human breast cancer and melanoma on NK cells were identical (Liu et al. 2006). FasL- and TRAIL-expressing exosomes were also shown to induce apoptosis in tumor-specific activated T effectors (Abusamra et al. 2005). Treatment of immature mouse DCs with exosomes derived from breast cancer blocked maturation of DCs and stimulated prooncogenic cytokine response, as indicated by increase of IL-6 and activation of STAT3 pathway (Liu et al. 2006, reviewed in: Zhang et al. 2012). Tumor exosomes containing PGE_2 and TGF-β also promoted MDSCs to decrease T-cell cytotoxicity (Xiang et al. 2009). *In vivo* studies showed the presence of exosomes in cancer patients' sera, the fact that was correlated to the increased number of Tregs. It could be possible that exosomes containing suppressory cytokines IL-10 and TGF-β were involved in Tregs expansion in these patients, as a similar phenomenon was described in *in vitro* studies (reviewed in: Whiteside et al. 2011). Therefore, exosomes may be viewed as modulators of immune response and inducers of both local and peripheral tolerance toward tumor (reviewed in: Valenti et al. 2007). However, some studies demonstrated that DC-derived exosomes could stimulate antitumor T-cell responses and activate NK cells. Probably different composition of tumor-derived and DC-derived exosomes could be responsible for that discrepancy (reviewed in: Zhang et al. 2012).

1.5 Conclusions

Cancers are capable not only to escape from host immune surveillance, but also to modulate it in order to improve conditions for tumor growth and metastasizing. To achieve this, tumors use a complex and diversified combination of mechanisms. Therefore, treatment based simply on either enhancement of tumor antigenicity or patient's immune response, although effective in many circumstances, still lacks satisfactory accuracy. Management based on multidirectional disorganization of tumor growth or a combination of biological and chemical drugs seems to show more optimistic results; however, a much deeper knowledge about tumor biology is needed to achieve more satisfactory results.

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