The microenvironment as determinant of testicular germ cell tumor biology

Timmer, Albertus

TESTICULAR GERM CELL TUMORS

As reviewed in **Chapter 1**, testicular germ cell tumors can be divided into seminomas, composed of neoplastic gonocytes, and nonseminomas, pluripotent tumors which can be considered as the neoplastic counterpart of early embryos. The germ cell origin of these tumors is firmly established, and current concepts consider intratubular germ cell neoplasia as the preinvasive stage of all adult testicular germ cell tumors with the possible exception of spermatocytic seminoma. Evidence is emerging that seminomas and nonseminomas are histogenetically related and represent stages in tumor progression of testicular germ cell tumors. Progression of intratubular germ cell neoplasia to seminoma and/or nonseminoma is accompanied by significant changes in ploidy and karyotype. The inherent aggressiveness of the histological subtypes largely determines the heterogeneity of testicular germ cell tumors with respect to clinical presentation and reaction to therapy. Yet, (epigenetic) factors that control phenotypic expression, and thus the biology of the histological subtypes, are largely undefined. The choice of therapy in testicular germ cell tumors depends on the histology of the primary tumor (seminoma versus nonseminoma), clinical stage, and the anticipated biological stage. The latter may be extrapolated from clinical, pathological, biological, and genetic parameters. Although testicular germ cell tumors have become a model for curable cancer, presently about 20% of all patients with a testicular germ cell malignancy will succumb due to the growth of residual tumor resistant to chemotherapeutic drugs.

INTEGRINS AND EXTRACELLULAR MATRIX PROTEINS IN THE BIOLOGY OF TESTICULAR GERM CELL TUMORS

Normal testis and testis with intratubular germ cell neoplasia

In **Paragraph 2.1.2** a review is given on the presence and function of extracellular matrix (ECM) proteins in the developing and mature testis. Similar to morphogenetic processes in other organ systems it appears that ECM proteins play a pivotal role in testicular development. For example, the selective distribution of fibronectin along the migration pathway of primordial germ cells suggests that migration of primordial germ cells is at least in part dependent on their interaction with fibronectin. The onset of testicular cord formation is associated with marked changes in the distribution of ECM proteins. However, presently it is not yet clear whether cell-matrix interactions provide the initial signal that activates the morphogenetic cascade or whether initially soluble factors are involved. The coordinated interaction of both myoid cells and Sertoli cells is essential for the formation of the basement membrane (BM) that surrounds testicular cords and, later on, the seminiferous tubules. Conversely, in the adult testis normal seminiferous tubule function including spermatogenesis seems to be dependent on the cooperative action of Sertoli and myoid cells, in which the tubular BM plays a essential role. In atrophic tubules and in intratubular germ cell neoplasia the lamina propria and tubular BM were thickened with invaginations of the BM into the tubular lumen (**Chapter 2.2**). This increase in the deposition of ECM proteins suggest that the balance between production and degradation of BM constituents is lost, either by increased deposition or by decreased degradation of BM components. Yet, at this moment it is impossible to determine whether the impaired spermatogenesis is the trigger that leads

to increased deposition of ECM proteins or that the impaired spermatogenesis is secondary to the changes that occur in the tubular BM. In tubules containing intratubular germ cell neoplasia these changes may be related to a disregulation of testicular homeostasis, rather than to the presence of malignant intratubular germ cells per se, as similar abnormalities are observed in a variety of local and systemic pathological conditions that lead to tubular atrophy and hyalinization. Finally, in tubules with impaired spermatogenesis, Sertoli cells have been described to coexpress vimentin and keratin [1], a feature that is characteristic for immature prepuberal Sertoli cells [2]. By comparing the integrin profile of foetal and prepuberal Sertoli cells with Sertoli cells in atrophic and intratubular germ cell neoplasia containing tubules, one might elucidate whether increased expression of $\alpha 3$ and $\alpha 6$ integrin subunits on Sertoli cells in these abnormal tubules is a sign of immaturity.

Integrins in the biology of malignant tumors

The role of the integrin-matrix interactions in the biology of malignant tumors in general is discussed in **Paragraph 2.1.3**. Upon malignant transformation and subsequent tumor progression the pattern of integrin expression changes and becomes disorganized. However, lineage specific integrin distribution patterns may be conserved in malignant tumors, and occasionally reexpression of embryonal integrins may be observed. Compared to their nontumorigenic counterparts malignant tumors in general show a tendency to express reduced levels of integrins, although in some tumors integrins may be upregulated, or expressed de novo. Functionally, integrins may have inhibitory as well stimulatory effects on tumor cell growth, that in part may be related to conferral of resistance to anoikis (apoptotic cell response after detachment of cells from the ECM) or rescue from programmed cell death by ECM-mediated ligation of integrins present on the tumor cell surface. Furthermore, integrins may inhibit as well as promote the formation of metastases, and can play different roles at different metastatic stages. In line with current concepts on metastasis formation it appears that the effects of integrins on tumor growth and metastasis formation are not necessarily linked. Thus, although the redundancy in integrin-ligand interactions is enormous, experimental data point to different roles for specific integrins at different stages of the metastatic cascade. The association of specific integrin subunits with grade, stage, disease-free survival and overall-survival supports the view that integrins may be used as biological parameters that aid in the evaluation of the risk for metastasis formation and the choice of adequate therapy.

Seminoma

Data on the expression of integrin subunits and distribution of ECM proteins in intratubular germ cell neoplasia, seminoma and nonseminoma (**Chapters 2.2 and 2.3**) are concordant with data reviewed in Paragraph 2.1.3. Malignant intratubular germ cells showed a strong, nonpolarized expression of the $\alpha 3$, and $\alpha 6$ integrin subunits, and a weak expression of the ß1 integrin subunit. Progression of intratubular germ cell neoplasia to invasive seminoma was associated with loss of $\alpha 3$ integrin subunit expression. In primary seminomas the $\alpha 5$ integrin subunit was weakly expressed in all stages. All tumors showed a strong expression of $\alpha 6$ and ß1 integrin subunits. Compatible with degradation of BM components, in intratubular germ cell neoplasia the tubular BM revealed gaps besides thickened and irregular parts. Based on the distribution of collagen type I, 3 types of stromal reaction could

be recognized. Since differences between the three types of stromal reaction were partly relative, they may represent different stages in the ongoing process of fibrovascular stroma formation. Small band-like structures resembling BMs were randomly distributed in all primary seminomas and metastases of primary seminomas. These structures may well represent tubular remnants, not degradated by proteolytic enzymes. However, the linear distribution of BM components at the interface of tumor cells and stroma, and the presence of these structures in metastatic lesions suggests that BM components in seminomas may also be newly deposited.

The heterogeneity in integrin subunit expression and distribution of ECM proteins suggest a dynamic interplay between seminoma cells and host cells. However, the contribution of both cell populations to the formation of the ECM has to determined. It seems likely that seminoma cells themselves do not contribute significantly to the formation of the ECM as cytoplasmic staining for ECM proteins is not evident (unpublished observations, [3]). Whatever the origin of the ECM in seminomas, the interaction of seminoma cells with the microenvironment is likely to interfere with processes such as cell survival, proliferation, and migration. The observation that seminoma cells enter an apoptotic pathway, when kept in vitro in the absence of a specific matrix [4], is in line with this hypothesis, and future studies will have to elucidate whether this response can be viewed as anoikis. In contrast to the former, integrin-matrix interactions may also have an adverse effect on the invasiveness and metastatic competence of seminoma cells. Experimental studies suggest that the adhesiveness of primordial germ cells to fibronectin in part determines the motility of these cells. E.g., the switch from nonmigratory to migratory stages of primordial germ cell migration coincides with a decreased adhesion of primordial germ cells to fibronectin (Paragraph 2.1.1). Similarly, and analogous to the adhesion of intratubular cells to the tubular BM, adhesion of seminoma cells to BM-like structures and the interstitial matrix may hamper their migration and bear on the development of metastases. Interestingly, malignant intratubular germ cells and invasive seminomas express the same integrins (α3, α5, α6, and ß1) as nonmigratory murine primordial germ cells. As primordial germ cell migration is a highly conserved process in different species (Paragraph 2.1.1) one might speculate that human primordial germ cells use the same set of integrins as their murine counterparts. Future studies will have to elucidate whether the (adhesive) mechanisms used by malignant intratubular germ cells and seminoma cells to survive, proliferate and migrate, are analogous to the ones used by their nontumorigenic precursors and counterparts, e.g., human primordial germ cells and gonocytes respectively. Of special interest is the role of c-kit-stem cell factor interactions as c-kit is expressed by human primordial germ cells as well as malignant intratubular germ cells and seminoma cells [5,6], and has been shown to modulate the (integrin-mediated) adhesiveness of hematopoietic cells to substrates coated with ECM proteins [7,8]. Similarly, one might speculate that binding of stem cell factor to c-kit on malignant intratubular germ cells and seminoma cells may modulate the expression and function of integrins on the tumor cell surface.

Nonseminoma

In **Chapter 2.3** results are presented on the expression of integrin subunits and distribution of ECM proteins in 34 testicular nonseminomas. In line with the epithelial nature of embryonal carcinoma cells are the weak expression of epithelial α2 and α3 integrin subunits, and the variable increased density of the α6 integrin subunit at the interface of

tumor cells and the stroma. Putatively, embryonal carcinomas with features of ongoing epithelial differentiation may behave differently, e.g., are associated with less malignant properties. Support for this case can be found from studies in which testicular germ cell tumors with an inherent capacity for somatic differentiation, apparent from a teratoma component, are associated with a better prognosis (Chapter 1). In contrast to highly invasive embryonal carcinoma, mature teratomas are supposed to be noninvasive and nonmetastatic. Concordantly, mature teratomas are composed of a variety of highly differentiated tissues derived from all three germ layers. Despite this benign phenotype, teratomas are in fact malignant as illustrated by the presence of marked genetic abnormalities, and the ability of residual mature teratoma to grow and progress to a non-germ cell malignancy (Chapter 1). Similar to their nonneoplastic counterparts, in teratoma each tissue has its own characteristic integrin profile, suggesting that in teratoma conserved presence of integrins may contribute to the high degree of differentiation and in this way to the indolent biological behavior of this histological subtype. Like teratomas, yolk sac tumors may also be composed of different histological subtypes [9]. Yet, in our study the number of yolk sac tumors was to limited to draw conclusions on the relation of integrin subunit expression and histological subtype. In choriocarcinomas syncytiotrophoblastic giant cells as well as cytotrophoblastic cells demonstrated a complex integrin phenotype. Most of the integrin subunits were shared by both cell types, although differences in staining intensity were observed. Compared to normal placental cytotrophoblastic cells, in choriocarcinoma cytotrophoblastic cells showed aberrant expression of integrin subunits, putatively leading to a disbalance between cell-matrix interactions that promote invasion (collagen/laminin-α1ß1 integrin interactions) and those that restrain invasion (fibronectin-α5ß1 interactions) of normal cytotrophoblastic cells. The differences in distribution of ECM proteins between the different histological subtypes were relative. Variable in vitro synthesis of ECM proteins by different histological subtypes suggests an in part lineage dependent tumor cell origin of ECM proteins in vivo [10-13]. Investigation of ECM protein isoform distribution, for example by using antibodies directed against the different chains of the laminin molecule [14], may reveal in addition to quantitative differences, qualitative differences in distribution of ECM proteins, that can be expected to be relevant to the biology of these tumors [15,16]. Interestingly, in vitro growth of embryonal carcinoma cells can be supported by ECM proteins secreted by yolk sac tumors [12]. Similarly, and analogous to paracrine support of neighboring cells during the early stages of embryogenesis [11] one might hypothesize that paracrine support of one particular germ cell tumor to another is relevant to the biology of testicular germ cell tumors as well.

Of particular interest is the remaining question how differentiation in testicular germ cell tumors is regulated. Based on several studies, a prominent role for genetic factors can be recognized as several tumor suppressor genes and oncogenes show a histological subtype restricted expression pattern, that may be determined by tumor progression related loss or gain of genes essential to growth and differentiation [17,18]. The imprinting status of the genome in testicular germ cell tumors is supposed to be dependent on the maturation stage of the primordial germ cell at the time of initiation. In fact, by regulating which part of the genome will be transcribed, genomic imprinting may determine whether in testicular germ cell tumors differentiation proceeds along the germinal, embryonal, or extraembryonal lineage (Chapter 1). The extensive differentiation of human embryonal carcinoma cells after subcutaneous injection of nude mice (Chapters 4.1 and 4.2), and the derivation of distinct cell types upon application of different differentiation inducers [19,20] suggest that the phenotype of human testicular germ cell tumors is dependent on the cooperative action of multiple factors present

in the microenvironment. Indeed, the heterogeneity of testicular germ cell tumors with respect to integrin subunit expression suggests that cell-matrix interactions are involved in differentiation of these tumors. Yet, whether integrins contribute actively to differentiation by inducing and/or repressing histological subtype specific gene expression (outside-in signalling), or whether expression of integrins is a reflection of the process of differentiation itself (inside-out signalling) is to be determined. In a recent study, morphological differentiation of murine F9 embryonal carcinoma cells was shown to be prevented by abrogation of ß1 integrin-ECM interactions [21]. Analogously, one might hypothesize that the morphological changes that coincide with tumor progression of intratubular germ neoplasia to invasive nonseminoma are integrin mediated as well. Obviously, to confirm this hypothesis, seminoma cell lines are needed in which seminoma cells can be reprogrammed to pluripotency. Besides cell-matrix interactions other epigenetic factors may contribute to testicular germ cell tumor differentiation. Among these growth factors/cytokines, hormones, vitamins, and cell-cell interactions can be considered to be the most important. Intratumoral variation of these factors, and of metabolic substrates, PH and oxygenation, gives a selective pressure that may lead to heterogeneity, and further contributes to differentiation of these tumors [22-24].

In conclusion, the developmental potential of testicular germ cell tumors seems to be restricted by the (tumor progression related) genetic make up of the tumor and the imprinting status of the genome. Yet, to express the differentiated phenotype microenvironmental factors are needed. This regulatory model assumes that differentiation in testicular germ cell tumors is highly regulated, and therefore susceptible to the differentiation inducing activity of agents like retinoids.

DIFFERENTIATION INDUCTION AS PART OF ANTI-CANCER THERAPY IN MURINE AND HUMAN TERATOCARCINOMAS

A major challenge in the treatment of patients with testicular germ cell tumors is the cure of patients with tumors that are inherently resistant or have become resistant to chemotherapeutic drugs. Salvage therapies have been developed and are under investigation. At the worst still 20% of patients with advanced nonseminomas can not be cured. Potentially retinoids might play a role in better treatment results in these groups. Combination of chemotherapy and induction of (terminal) differentiation by means of retinoids might theoretically be fruitful as the mechanisms of action are quite different: tumor cell kill and in addition reversion of malignant phenotype of (chemotherapy resistant) tumor cells into terminally differentiated nonproliferating, noninvasive cells.

Murine teratocarcinoma

In **Chapter 3** the combination of a cytotoxic drug and a differentiation inducing agent in vivo in three murine teratocarcinoma models with different levels of spontaneous somatic differentiation (E86-379 (moderate differentiation); NF-1 (poor differentiation); MH-15 (no differentiation)) is discussed. Cisdiamminedichloroplatinum(II) (CDDP) was used as a cytotoxic drug and all-trans retinoic acid was used as differentiation inducing agent. The untreated tumors grew progressively. CDDP alone prevented tumor growth. The combination

of all-trans retinoic acid and CDDP gave a significant further reduction of tumor size as compared with either all-trans retinoic acid or CDDP alone. All-trans retinoic acid as differentiation inducing agent increased the percentage of differentiated residual tissue, but only in tumors with the capacity of spontaneous somatic differentiation (NF-1 and E86-379). The nullipotent MH-15 tumor was not induced to differentiate, and thus did not give rise to residual mature teratoma. Rather than having an adverse effect, it turned out that all-trans retinoic acid alone tended to reduce tumor growth in all models, however, only significant reduction was found in the MH-15 tumors. This indicates that, similar to the effect of 13-cis retinoic in xenografts of Tera-CP (Chapter 4.2), the effect of all-trans retinoic acid in MH-15 tumors probably was more a result of retinoid-induced growth inhibition than a effect of induction of differentiation. Thus, in murine teratocarcinomas the use of all-trans retinoic acid as a differentiation inducing agent indeed increases the percentage of differentiated residual tumor, but only in tumors with the capacity of spontaneous somatic differentiation.

Human teratocarcinoma

Acquired resistance to CDDP is one of the major problems encountered in the therapy of testicular germ cell tumors. The mechanisms contributing to CDDP resistance are diverse, and include reduced drug accumulation and increased detoxification in the cytoplasm. In the cell nucleus, decreased DNA accessibility and DNA repair may play a role [25,26]. **Chapter 4.1** describes a 3.7 fold CDDP-resistant subline (Tera-CP) of NTera/2D1 (Tera), an human embryonal carcinoma cell line with the capacity to differentiate in vitro as well as in vivo. In vitro both cell lines mainly consisted of embryonal carcinoma cells. For this reason, in Tera-CP CDDP-resistance seems not to be due to a selection of somatically differentiated cells, as is observed in residual lesions of patients treated for a testicular nonseminoma with an inherent capacity for somatic differentiation apparent from a teratoma component (Chapter 1). Tera-CP showed an 1.4-fold increased glutathione (GSH) level, a 1.5-fold increased glutathione S-transferase (GST) activity, and a 1.4-fold increased GST-π expression compared to Tera. The decreased binding of CDDP to DNA in Tera-CP may, in the absence of accumulation defects and in the presence of normal activity of DNA repair enzymes (thymidilate synthase, topoisomerase I and II) be due to an increased efficiency of the detoxifying system. Based on the response of Tera and Tera-CP to a single intraperitoneal dose of 5 mg/kg body weight, an in vivo resistance factor of 2.8 could be calculated. In new cell lines derived from xenografts of Tera and Tera-CP, CDDP sensitivity, GST activity and GSH level corresponded with their sensitivity and resistant origin. Thus, Tera-CP is a model of in vitro and in vivo CDDP resistance with the GSH/GST detoxifying system as an important mechanism. The close correlation of in vitro and in vivo degree of resistance excludes a role for in vivo active, tumor associated factors that influence the pharmacokinetics of CDDP in this nude mouse model, a phenomenon described for a model with in vivo acquired resistance [27]. Generally, in vitro selection of sublines resistant to chemotherapeutic drugs is accompanied by a decrease in malignant potential, and this phenomenon is referred to as "reverse transformation" [28]. However, compared to the CDDP sensitive parental cell line Tera, the CDDP-resistant subline Tera-CP demonstrated an increased tumorigenicity (Chapter 4.2). Although in vitro both cell lines showed the same propensity to differentiate [29], in vivo the percentage of differentiated tissue in Tera is higher than in Tera-CP, and this difference may explain at least in part the increased tumorigenicity of Tera-CP cells.

In **Chapter 4.2** data are presented on the potential of the combination of a differentiation inducing agent and CDDP treatment, in comparison to each drug alone in xenografts derived from Tera and Tera-CP. Based on the more favorable in vivo pharmacokinetics and the expectation of biologically comparable results, in the present study 13-cis retinoic acid was used as a differentiation inducing agent instead of all-trans retinoic acid. CDDP sensitivity of tumor cells in vivo was not negatively influenced by (pre)treatment with 13-cis retinoic acid. Neither in Tera nor in Tera-CP an increased tumor reduction could be observed in mice treated with CDDP followed by 13-cis retinoic acid compared to the mice treated with CDDP alone. This was in contrast with in vivo potentiation of CDDP cytotoxicity by all-trans retinoic acid in tumors of murine embryonal carcinoma cells with an inherent capacity to differentiate (Chapter 3). This discrepancy could not be due to a complete lack of 13-cis retinoic acid-effect, as in the Tera-CP tumors with only 13-cis retinoic acid a tumor reduction of 20% was achieved. Analogous to the development of clinically favorable residual mature teratoma after chemotherapy for testicular germ cell tumors with an inherent capacity for somatic differentiation (Chapter 1), in the CDDP sensitive Tera tumors with the more extensive differentiation, differentiated cells with inherent resistance to CDDP seemed to survive treatment selectively. In the Tera-CP tumors with acquired CDDP resistance, with less spontaneous somatic differentiation, not only differentiated cells but also embryonal carcinoma cells were present after treatment. The latter gave rise to regrowth of the tumor. Thus, residual lesions of Tera and Tera-CP tumors after treatment with CDDP showed similarities to inherent (CDDP resistant somatically differentiated tumor cells) and acquired CDDP resistance (CDDP resistant undifferentiated embryonal carcinoma cells), as is encountered in the clinical situation. In Tera-CP, addition of 13-cis retinoic acid to CDDP treatment increased differentiation of post-therapy residual tumor cells, thus reducing the invasiveness and metastatic competence of this tumor residue. However, in Tera and Tera-CP differentiation was incomplete and immature. As in vitro embryonal carcinoma cells can be induced to differentiate terminally into nonmitotic neuronal cells [30], one might speculate that the treatment schedule used in our experiment was inappropriate. In order to improve antitumor activity, simultaneous administration of 13-cis retinoic acid and CDDP should be considered for future studies. Regrowth of murine teratocarcinomas after the dose reduction of CDDP (Chapter 3), and the inability of 13-cis retinoic acid to prevent regrowth of Tera-CP tumors when the effect of CDDP had vanished are further arguments for simultaneous administration of CDDP and 13-cis retinoic acid. Finally, enhancement of the effect of 13-cis retinoic acid can be reached by adding cytokines, or other biological response modifiers to the treatment regimen [31,32]. Future studies will have to reveal if these alternatives increase the response of embryonal carcinoma cells to retinoids. Finally, our recent data differ from earlier results [29], as in vitro pretreatment of Tera and Tera-CP with all-trans retinoic acid (and 13-cis retinoic acid, H. Timmer-Bosscha, personal communication) for 96 hours strongly prevented cytotoxicity induced by CDDP. Apparently, the response of both cell lines to retinoids is modulated by the microenvironment in which embryonal carcinoma cells reside.

The expression of many genes is altered after treatment with retinoids. Among these are genes that encode proteins that play a role in cell-matrix interactions (Chapter 1). In **Chapter 4.3** the expression of integrin (subunits) in the human embryonal carcinoma cell line Tera and its 3.7 fold CDDP resistant subline Tera-CP is discussed before and after treatment with 10^{-7}M all-trans retinoic acid for 96 hours. The α1 and α3 integrin subunits were weakly

expressed in less than 5% of the tumor cells. Both cell lines showed uniform expression of α5, α6, αv, and ß1 integrin subunits. The αvß5 integrin was expressed by nearly all tumor cells. Neither in Tera nor in Tera-CP α2, α4, ß3, and ß4 integrin subunits could be detected. Although integrins expressed by Tera and Tera-CP are likely to play an active role in growth and differentiation, it is difficult to discern whether expression of integrin subunits simply is a tissue culture induced phenomenon, or alternatively reflects the degree of malignancy of the cell, and could be related to the degree and lineage of differentiation. No differences were observed in integrin (subunit) expression that might account for differences in growth and differentiation observed in vivo (Chapter 4.2). Moreover, it seems likely that integrins do not play a major role in cell functions operational in resistance to CDDP. However, the extensive differentiation observed in xenografts of Tera as well Tera-CP (Chapters 4.1 and 4.2) suggests that differentiation in both cell lines is dependent on multiple factors, present in the microenvironment. Hypothetically, the dissimilarity in genetic make up between Tera and Tera-CP may result in differences in cellular signal transduction pathways that only becomes apparent after engagement of the proper microenvironment. Upon application of all-trans retinoic acid the α4 integrin subunit was expressed de novo in less than 5% of the tumor cells, whereas increased staining intensity was noticed for the α5 integrin subunit, suggesting that in Tera and Tera-CP all-trans retinoic acid may exert its action by modulation of cell-matrix interactions. In vitro pretreatment of murine F9 embryonal carcinoma cells with all-trans retinoic acid resulted in a decreased tumorigenicity and a different pattern of metastasis formation of tail vein injected tumor cells [33], that at least in part may depend on modulation of the growth response of tumor cells to the local microenvironment, and changes in the (ß1 integrin mediated) adhesiveness of tumor cells [34,35]. Future studies will have to elucidate whether, similar to the effects of all-trans retinoic acid on murine F9 embryonal carcinoma cells, in Tera and Tera-CP retinoid-induced changes in integrin expression are related directly to modulation of growth, invasiveness, and metastatic competence of tumor cells.

CONCLUSIONS AND FUTURE PROSPECTS

It is conceivable to assume that parallels can be drawn between tumors cells and their nonneoplastic counterparts, as tumors can be considered as caricatures of normal tissue renewal and early embryogenesis [36,37]. Concordantly, compared to their nonneoplastic counterparts, in testicular germ cell tumors a partially conserved expression of integrin subunits was noticed, suggestive to be related to differentiation lineage and biological behavior (Chapters 2.2 and 2.3). However, processes such as invasion and development of metastases are highly regulated, and multiple factors are likely to contribute to the invasiveness and metastatic competence of tumor cells. The disregulation of multiple regulatory mechanisms operative during physiological processes determines the aggressiveness of a tumor [38,39]. From this point of view, the role of integrins in the biology of testicular germ cell tumors can only be fully understood if the relation between integrin-dependent and nonintegrin-dependent signal transduction pathways is elucidated.

In order to grow, invade, and metastasize, tumor cells have to elicit their own microenvironment or must become, to a certain degree, independent from their specific microenvironment. In agreement with this, in testicular germ cell tumors ECM protein distribution was heterogeneous, and suggestive to be related to the biology of seminomas as well as nonseminomas. However, the mechanisms responsible for this heterogeneity have to