Mechanisms of Ewing sarcoma metastasis : biochemistry and biophysics

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Chapter 1

Introduction

1.1 Ewing sarcoma

Ewing sarcoma (ES) is a special type of bone cancer, first described by Dr. James Ewing in his paper 'Diffusive endothelioma of bone' (Ewing, 1972; Appendix 1). Gathered under a common name and further referred as Ewing sarcoma (ES for short) this group of aggressive neoplasms includes Ewing sarcoma of bone, extra-skeletal Ewing sarcoma, Askin tumor and peripheral primitive neuroectodermal tumor (Delattre et al., 1994; Iwamoto, 2007; Potratz et al., 2012). Today Ewing sarcoma represents the second most common bone cancer among adolescents and young adults (Delattre et al., 1994; Lamhamedi-Cherradi et al., 2014; Ludwig, 2008). About 80% of the patients diagnosed with Ewing sarcoma are younger then 20 years old. It is rarely diagnosed in patients older then 30-40 years old (Iwamoto, 2007; Karosas, 2010). To the present day the oldest patient documented case of Ewing sarcoma belongs to a 85 years old female patient (Monument et al., 2015).

Ewing sarcoma predominantly develops in bone with the most common sites being the long bones (Fig. 1.1; Barker et al., 2005; Iwamoto, 2007; Karosas, 2010; Ludwig, 2008; Potratz et al., 2012). In soft tissues Ewing sarcoma may develop in older patients. The earliest symptom of ES is pain, followed by swelling and fever (Iwamoto, 2007; Karosas, 2010). Ewing sarcoma is often overlooked (Karosas, 2010; Nedelcu et al., 2014), the diagnosis frequently is delayed by weeks and up to years (Ludwig, 2008; Nedelcu et al., 2014). The increased time to ES diagnosis is primarily associated with older age of patient and tumor site (Brasme et al., 2014). Tumor sites associated with increased time to diagnosis are skull, ribs, vertebrae, limbs, pelvis, and hand or foot (Brasme et al., 2014). Innumerous studies together with the development of chemotherapy (1962) and multi-modal cancer-treatment protocols increased the 5-years survival rate for Ewing sarcoma patients with localized tumor from 10% to 70% (Iwamoto, 2007; Karosas, 2010; Lamhamedi-Cherradi et al., 2014; Liebner, 2015; Potratz et al., 2012). Patient survival and metastasis formation was shown to be not significantly associated with time to diagnosis (Brasme et al., 2014). However, in more then half of the diagnosed Ewing sarcoma cases micrometastases are presumed to be present (Lamhamedi-Cherradi et al., 2014). Contrary to the positive achievement in treatment of localized tumors, the long-term (5-years) survival for Ewing sarcoma patients with metastasis, however, remain below the 30% mark (Lamhamedi-Cherradi et al., 2014; Ludwig, 2008;

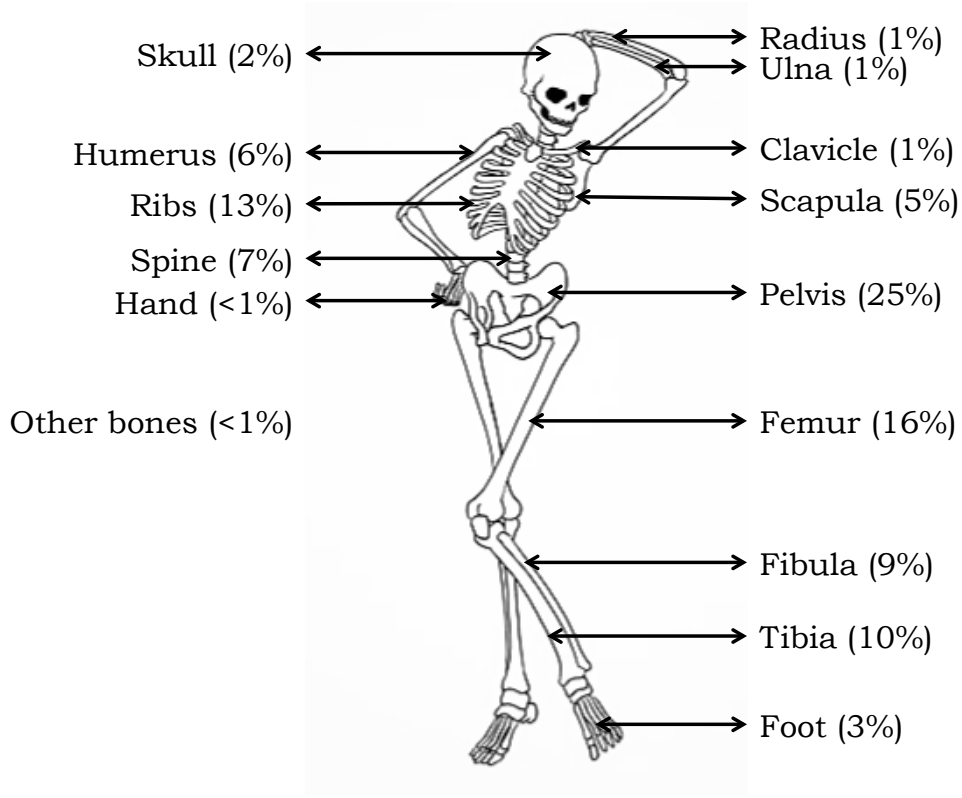

Figure 1.1
Representation of Ewing sarcoma distribution sites in human skeleton.

Potratz et al., 2012). Hence, a better understanding of the processes underlying Ewing sarcoma metastasis deserves additional attention.

1.2 Ewing sarcoma's origin

First Ewing sarcoma cell lines were established in 1970's. However, often cells put in culture were initially mistakenly described as neuroblastoma (Schlesinger et al., 1976), rhabdomyosarcoma (Giard et al., 1973), lymphoid cells or others. Only later characterized as Ewing sarcoma (Martinez-Ramirez et al., 2003; Whang-Peng et al., 1986), these cells raised a question of a common cell origin of Ewing sarcoma. There is evidence indicating that Ewing sarcoma derives from mesenchymal cells (Kovar, 2014; Pagani et al., 1995). Other studies show that Ewing sarcoma originates from pluripotent cells with blocked differentiation (Kovar, 2005). Lately an increasing number of reports of cellular studies

suggest a neuronal origin of: CADO (Kodama et al., 1991), SS-ES-1 (Hatori et al., 2006) and other cell lines (Cavazzana et al., 1987). However, poor differentiation and the stem cell like phenotype of Ewing sarcoma cells keep the cellular origin of Ewing sarcoma unresolved (Sand et al., 2015). A better understanding of Ewing sarcoma origin would, hence, potentially uncover mechanisms that control ES growth, progression and metastasis leading to novel therapeutic strategies.

1.3 EWS/FLI fusion protein

Histologically Ewing sarcoma is identified as a small round cell tumor. Over 90% of tumors are positive for the surface antigen MIC2 (CD99) (Potratz et al., 2012). Cytogenetically Ewing sarcoma is characterized by a specific chromosomal translocation t(11;22) (Aurias and Desmaze, 1992; Fraccaro et al., 1980; Iselius et al., 1983; Turc-Carel et al., 1988). In 80% of the cell lines and 90% of the primary Ewing tumors this translocation is detectable (Dockhorn-Dworniczak et al., 1994). Thus, presence of the t(11;22) translocation became the accepted hallmark in the differential diagnosis and prognosis of Ewing sarcoma (Dockhorn-Dworniczak et al., 1994; Turc-Carel et al., 1988). There is a slight variation in breaking points of this reciprocal translocation. The most common, occurring 85% of the time, involve band q12 and band q24 of chromosome 22 and chromosome 11, t(11;22)(q24;q12) (Zucman et al., 1992). Translocation results in the expression of the fusion protein EWS-FLI-1 (Delattre et al., 1994; Dockhorn-Dworniczak et al., 1994; Ludwig, 2008). Less common, occurring 5-8% of the time, is the fusion protein EWS-ERG and, occurring in less than 1% of the time, the fusion proteins EWS-ETV1, EWS-EIAF, or EWS-FEV (Delattre et al., 1994; Dockhorn-Dworniczak et al., 1994) . The fusion protein EWS/FLI-1 was shown to act as a strong transcriptional activator (May et al., 1993; Sand et al., 2015) that interferes with the expression of many genes. Key proteins of various pathways like IGF-1R, mTOR, MAPK, PI3K/Akt, EGFR, VEGF and others were found to be disregulated (Ludwig, 2008). For instance, influenced by EWS-FLI-1, the IGF-1R pathway appears to be constitutively activated in Ewing sarcoma and subsequently emerge as a key player in the malignant transformation (Ludwig, 2008). Moreover over-expressed *in vitro* or *in vivo*, EWS-FLI-1 promotes cell growth and facilitates susceptibility to chemotherapy (Ludwig, 2008). Hence, EWS/FLI potentially

plays a central role in Ewing sarcoma progression and metastasis.

Another evidence supporting the above conclusion is a high involvement of EWS/FLI in regulation of the chemokine receptor CXCR4. CXCR4 was shown to correlate with Ewing sarcoma metastasis (see below) and poor prognosis for patients (Bennani-Baiti et al., 2010; Kim et al., 2010).

1.4 CXCR4

In 1994 for the first time a leukocyte-derived seven-transmembrane domain receptor (LESTR) cDNA was isolated. The high expression of LESTR in white blood cells was suggested to play a role in inflammation. (Loetscher et al., 1994). In May 1996 using a cDNA cloning strategy an HIV-1 cofactor was isolated, which supposedly was identified as G-protein coupled receptor with seven-transmembrane domain structure. This cofactor was shown to promote HIV-1 fusion and virus entry to CD4+ cells. Therefore, the protein was designated as Fusin. (Feng et al., 1996). The discovery of Fusin was a real breakthrough in HIV-1 research (Broder and Dimitrov, 1996; Cohen, 1996). LESTR and Fusin happened to be the same receptor. In August 1996 the stromal-derived factor-1 (SDF1, later designated as CXL12) has been reported as specific chemoattractant ligand to LESTR/Fusin (Bleul et al., 1996; Oberlin et al., 1996). Since then the previously orphan chemokine receptor was designated as CXC chemokine receptor 4, short CXCR4 (Bleul et al., 1996). SDF-1 binding to CXCR4 was shown to be a strong inhibitor of infection by HIV-1 (Bleul et al., 1996), due to the role of the CXCR4 ligand-binding domain in HIV-1 entry (Picard et al., 1997). In 2001 the involvement of CXCR4 in breast cancer metastasis was suggested (Muller et al., 2001). This initial association was subsequently followed by reports, proving the role of CXCR4 in metastasis of a broad spectrum of cancer types. Up to date, the function of CXCR4 as co-receptor for HIV entry and its role as cancer metastasis promoter made it the best-studied human chemokine receptor (Furusato et al., 2010). Nevertheless, a lot of molecular details of CXCR4 function stayed yet undiscovered.

1.4.1 CXCR4 structure

The chemokine C-X-C motif receptor 4 (CXCR4) is one of ∼20 described human chemokine receptors. CXCR4 belong to the superfamily of G-

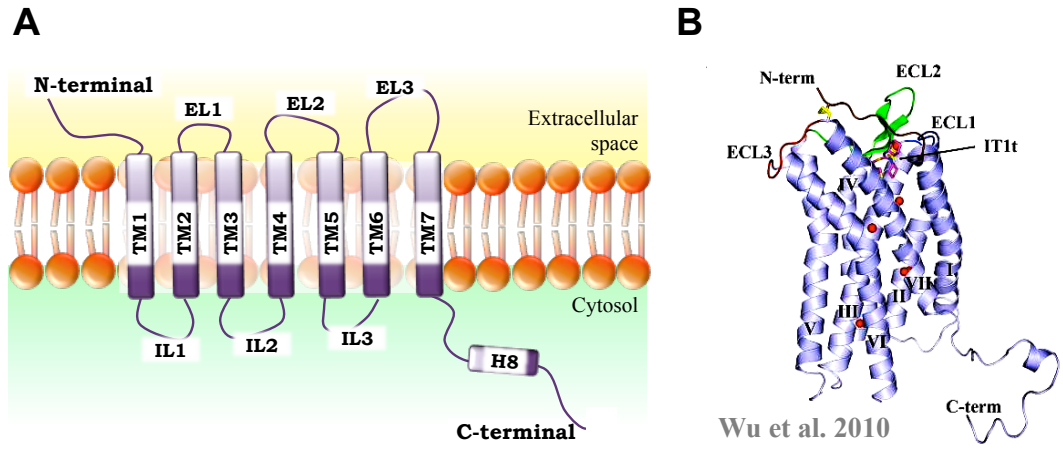

Figure 1.2
CXCR4 structure. ***A.*** *A schematic representation of the structure of any G-protein coupled receptor, consisting of an extracellular N-terminus, three extracellular loops (EL), seven transmembrane (TM) α-helixes, three intracellular loops (IL), an amphipathic helix H8 and an intracellular C-terminus.* ***B.*** *Crystal structure of chemokine receptor CXCR4 (from Wu et al., 2010).*

protein coupled receptors (GPCRs). Constituting about 800 genes of the human genome GPCRs share a common seven-transmembrane α-helix protein structure (Fig. 1.2). CXCR4 is one of the few GPCRs and the only chemokine receptor of which the structure was successfully resolved by X-ray crystallography (Wu et al., 2010; Fig. 1.2B). The structure showed a remarkable resemblance with the resolved structure of rhodopsin (Palczewski et al., 2000) and predicted earlier structures of other GPCRs (Costanzi et al., 2009). The common structure of G-protein coupled receptors consists of an extracellular N-terminus, three extracellular loops (EL1-EL3), seven transmembrane α-helixes (TM1-TM7), three intracellular loops (IL1-IL3), a small intracellular amphipathic helix (H8) and an intracellular C-terminus (Fig. 1.2A). Some GPCRs, e.g. CXCR4, are lacking the amphipathic helix. Each part of GPCR has its recognized role during receptor activation (Rajagopalan and Rajarathnam, 2006; Rosenkilde et al., 2000; Venkatakrishnan et al., 2013). The N-terminus and the EL are responsible for specific ligand recognition and modulation of the ligand access. The C-terminus and the IL are responsible for modulation of receptor activity and downstream signaling. The TM region is a communication link between extracellular and intracellular part of the receptor.

1.4.2 Activation mechanism

CXCR4 ligand, CXCL12, binding is a two step process. Initial interaction between the ligand and the N-terminus lead to conformational changes of the receptor by which a stable interaction of the ligand with the exposed binding pocket in CXCR4 occurs (Busillo and Benovic, 2006). Disulphide bridges in the extracellular region contribute to receptor stability (Venkatakrishnan et al., 2013). Agonist binding disrupts the existing intramolecular interactions within CXCR4 and promotes formation of interactions, which result in energetically favorable conformational rearrangements of the receptor (Wess et al., 2008). The conformational rearrangements mainly involve the transmembrane region of the receptor and can be summarized as: (1) small local structural changes in the TM5; (2) relocation of TM3 and TM7; (3) translation/rotation of TM5 and TM6 (Venkatakrishnan et al., 2013). TM7 together with TM6 are bending inwards towards TM3 (Hoffmann et al., 2008) resulting in the formation of cross-linking disulfide bonds between the cytoplasmic ends of TM3 and TM7 (Wess et al., 2008). TM helix 6 undergoes the largest movement (Hoffmann et al., 2008; Wess et al., 2008) resulting in reorientation of its cytoplasmic end. It was demonstrated that the rotational movement of the cytoplasmic end of TM6 (Hoffmann et al., 2008) causes a conformational change of the third intracellular loop (Hoffmann et al., 2008; Wess et al., 2008). The conformational change in IL3 subsequently induces activation of G-protein dependent signaling pathways (Roland et al., 2003) as the main signal transduction mechanism.

1.4.3 CXCR4 signaling

Activation of CXCR4 triggered by its specific agonist CXCL12 is a complex process. Binding of CXCL12 to CXCR4 initiates receptor signaling through four different G_α subunits (Rubin, 2008), resulting in PCLβ-, Cdc42-, Akt-, Erk-, and Rho- dependent biochemical cascade activation. Additionally, G-proteins independent pathways are activated, resulting in C-terminal phosphorylation and activation of the β-arrestin pathway leading to receptor internalization and β-arrestin signaling, respectively (for a review see: Busillo and Benovic, 2006; Rubin, 2008; Teicher and Fricker, 2010). The filigreed regulation and precise control over these various pathways finally result in a ligand-specific cellular reaction including gene expression, cell proliferation, cell growth and cell migration. The

latter is the key outcome on CXCL12-dependent chemotaxis. Chemotaxis, the directional cell migration in a gradient of a ligand, is refered to as the central process during angiogenesis, embryogenesis, stem cell homing, as well as inflammation and cancer metastasis (Kryczek et al., 2007; Teicher and Fricker, 2010). Hence, CXCR4 is recognized as a chemokine receptor actively promoting cell migration during cancer metastasis.

1.4.4 CXCR4 in cancer metastasis

Breast, lung, ovarian, renal, prostate and other cancer cells were shown to express high levels of CXCR4. In breast cancer (Muller et al., 2001), prostate cancer (Wang et al., 2005), lung cancer (Phillips et al., 2003) and others elevated CXCR4 expression has been associated with metastatic disease and poor prognosis. Cancer cells exhibit expedited proliferation in response to CXCL12 stimulation, while in their healthy counterparts CXCL12-induced apoptosis was detected (Rubin, 2008). Thus, a model was proposed, suggesting a different kinetics of the CXCR4 signaling in cancer cells. The changed kinetics would cause overlap in G-protein dependent and independent pathways in such a way, that it results in the altered cellular response (Rubin, 2008).

CXCR4 was suggested to promote tumor progression by increased cell growth, induction of angiogenesis towards the tumor tissue and formation of metastasis (Berghuis et al., 2012; Jin et al., 2012). The interruption of CXCL12/CXCR4 signaling was shown to inhibit the metastatic processes (Krook et al., 2014; Muller et al., 2001; Vandercappellen et al., 2008). It was suggested that the high level of CXCL12 expression in tissues such as lungs, liver, lymph nodes and bone marrow defines them as main target sites for cancer metastasis driven by CXCR4 chemotaxis (Kim et al., 2010; Raman et al., 2007; Vandercappellen et al., 2008). Hypoxia, which typically accompanies tumor formation, was further suggested to induce tumor-specific CXCL12 expression, which potentially initiate angiogenesis towards the tumor (Kryczek et al., 2007; Raman et al., 2007). At the same time the decreased oxygen concentration within the tumor was shown to up-regulate CXCR4 expression further promoting cell migration out of primary tumor and metastasis.

1.5 Ewing sarcoma metastasis

Ewing sarcoma metastases occur at early stage of the tumor development. The primary sites of metastasis are lungs, other bones and/or the bone marrow. Metastasis to other tissues occurs in less then 1% of all cases. (Potratz et al., 2012). Thus, Ewing sarcoma exhibits a very unique metastasis phenotype, which is by far not well understood.

1.5.1 CXCR4 in ES metastasis

Similar to other cancer types, hypoxia results in up-regulation of CXCR4 expression in Ewing sarcoma. Additionally EWS/FLI1 result in up-regulation of CXCR4 expression. In turn, cells overexpressing CXCR4 display an increased chemotactic migration and invasion (Krook et al., 2014). Cell lines derived from ES patients with metastasis at diagnosis exhibited significantly higher expression level of CXCR4, compared to cell lines derived from patients with solid tumor only (Bennani-Baiti et al., 2010). The same increase of CXCR4 expression in metastasis compared to solid tumors was shown when speciments from the patients were analyzed (Jin et al., 2012). Thus, CXCR4 expression is correlated with Ewing sarcoma progression (Berghuis et al., 2012; Jin et al., 2012). Expression of CXCR4 in Ewing sarcoma is highly dependent on EWS-FLI1 (Bennani-Baiti et al., 2010) and is rapidly and reversibly modulated (Krook et al., 2014). The only other chemokine receptor regulated by EWS-FLI1 is the orphan-receptor CXCR7. As CXCR4 it binds to CXCL12, however CXCR7 activation is not followed by activation of G-protein signaling. Therefore, CXCR7 is considered to be a regulatory protein for CXCR4 signaling. Evidences suggest that CXCR7 plays a role in CXCL12 scavenging (Sun et al., 2010). However, hight CXCR7 expression in addition to high expression of CXCR4 in Ewing sarcoma was shown to be correlated with worse patient survival prognosis (Bennani-Baiti et al., 2010; Krook et al., 2014).

1.5.2 Other modulators of ES metastasis

Many other pathways and molecules were shown to contribute to Ewing sarcoma metastasis regulation. Chemokine receptor CXCR6 expression in tumor cells correlated with metastasis formation, while its ligand, CXCL16, was associated with localization of the metastasis in lungs

(Na et al., 2014). The orphan G-protein coupled receptor GPR64 was shown to specifically over-express in Ewing sarcoma. GPR64 promoted invasiveness and metastatic spread of Ewing sarcoma, while inhibition of GPR64 resulted in a reduced colony formation in vitro, and suppressed growth and metastasis of the tumor in vivo (Richter et al., 2013).

High levels of interleukin 6 expression in the tumor stroma of primary ES suggest a biological relevance of this cytokine in ES pathogenesis and, thus, mediate formation of metastases (Lissat et al., 2015). Pretreatment of Ewing sarcoma cells with stem cell factor (SCF) prevented metastasis formation in lungs in mice model (Landuzzi et al., 2000).

Also metalloproteinase (MMP) 2 and 9 activity correlats with ES cell invasion (Odri et al., 2014; Sainz-Jaspeado et al., 2010). Caveolin 1 (CAV1) involved in regulation of MMP and SPARC expression plays a key role in ES metastasis and lung colonization (Sainz-Jaspeado et al., 2010). Inhibition of the MMP 2 and 9 with zoledronic acid resulted in the decrease of spontaneous lung metastases dissemination from primary ES but not in the decrease the growth of prior lung metastases (Odri et al., 2014).

1.5.3 Anchorage-independent growth

Hypoxia condition in the tumor tissue induces accumulation of the hypoxia inducible factor (HIF)-1α in a big fraction of primary ES (Aryee et al., 2010). Ewing sarcoma cells adapt to hypoxia condition by modulating EWS-FLI1-dependent transcriptional signature (Aryee et al., 2010; Krook et al., 2014). Together with other micro-environmental stresses hypoxia promotes CXCR4-mediated Ewing sarcoma cell migration (Krook et al., 2014), invasiveness and anchorage-independent growth (Aryee et al., 2010). In turn, an anchorage-independent growth phenotype was shown to predict a severe metastatic potential of primary breast and lung tumors (Mori et al., 2009).

The adhesion of Ewing sarcoma cells is largely modulated by EWS-FLI1 (Chaturvedi et al., 2012; Chaturvedi et al., 2014). EWS/FLI reduce expression of zyxin and α_5 integrin (Chaturvedi et al., 2014), which causes the loss of organized actin stress fibers and focal adhesions (Chaturvedi et al., 2012), thereby promoting the loss of the cell spreading and enhance their capacity to grow in anchorage-independent conditions. Ewing sarcoma cells growing in anchorage-independent conditions form multicellular spheroids (Kodama et al., 1991; Landuzzi et

al., 2000; Paszek et al., 2005). While growing in spheroids cells show a considerably different phenotype compared to their monolayer counterparts. Ewing sarcoma spheroids show reduced proliferation, highly developed cell-cell junctions and anoikis resistance. Such phenotypic change is a prerequisite for the successful development of metastases as well as pronounced resistance to chemotherapy. (Strauss et al., 2010).

1.6 Cancer mechanics

1.6.1 Cellular stiffness

Besides cellular adhesion, expression of EWS/FLI largely changes the cytoskeleton of Ewing sarcoma cells. EWS/FLI expression induces a loss of well-defined stress fibers leading to a significant change in the cyto-architecture on ES cells (Chaturvedi et al., 2014). A depleted actin network was shown to be associated with a more aggressive phenotype in colon cancer (Palmieri et al., 2015). Mechanically the weakening of the cytoskeleton network leads to lower cell stiffness, which in turn increase their deformability and migratory capacity (Agus et al., 2013; Katira et al., 2013; Palmieri et al., 2015). Atomic force microscopy studies indicate that cancer cells are softer than normal cells (Hayashi and Iwata, 2015). Moreover, different stages of cancer can result in different changes of the mechanical phenotype of the cells (Katira et al., 2013). Metastatic colon cancer cells appear more modified, in terms of cell stiffness and actin network organization, compared to the cells from the primary tumor (Pachenari et al., 2014; Palmieri et al., 2015). The more aggressive cancer cells exhibit a decreased viscosity (Pachenari et al., 2014) and an increase in the non-specific adhesion toward substrates (Palmieri et al., 2015). The adhesion capacity of the cells is primarily attained through integrins, as integrins physically connect the cellular cytoskeleton to the extracellular matrix (ECM). Thus, an altered expression of integrins is thought to modulate cancer cells' phenotype. (Jansen et al., 2015).

1.6.2 Role of the extracellular matrix

Malignant tissues dynamically remodel the extracellular matrix (ECM) around them (Gill and West, 2014; Janmey et al., 2013; Jansen et al., 2015; Katira et al., 2013; Tung et al., 2015). Unlike individual cells, the overall tumor stiffness is increased compared to the normal tissue

(Gill and West, 2014). This effect is reached through more dense cellular packing and an excessive production of ECM (Gill and West, 2014; Tung et al., 2015). Increased tissue stiffness contributes to further tumor progression and, potentially, metastasis (Janmey et al., 2013; Sapudom et al., 2015; Tung et al., 2015). Some models suggest that the invasive morphology of cells is promoted through an increase of the stiffness of the cellular microenvironment (Katira et al., 2013). Mechanical stresses regulate cellular metabolism (Tung et al., 2015) and have a pronounced effect on cell proliferation (Janmey et al., 2013; Taloni et al., 2014). The ECM stiffness-dependent miRNA expression is mediating malignancy of breast epithelium (Mouw et al., 2014). Taken together, the cellular microenvironment and in particular the ECM has a significant impact on the metastatic profile of cancer cells.

1.7 Thesis outline

In this thesis a report on experimental work aiming for a better understanding of the mechanisms underlying Ewing sarcoma metastasis is presented. Two distinct mechanisms are investigated: (1) a biochemical approach in which the initial steps in the CXCR4 signaling cascade are followed, and (2) a biophysical approach in which the guidance of EW metastasis by the stiffness of their microenvironment is demonstrated.

In **Chapter 2** the molecular mechanism of chemokine receptor CXCR4 signaling in a model Ewing sarcoma derived cell line A673 is studied using the single-molecule imaging technique. Effects of activation-dependent dimerization, internalization and G-protein interaction are tested. Measurements in resting cells and cells stimulated with CXCL12 revealed an activation-dependent mobility change of CXCR4. The mobility change is shown to be associated with G protein-dependent and independent pathways. Data indicated a functional cross-talk between different biochemical cascades.

In **Chapter 3** the regulation of CXCR4 signaling in Ewing sarcoma is further addressed by investigation of the receptor interaction with the respective G-proteins. Two different G_{α}-subunits exhibited a differential coupling mode to CXCR4. Thereby, data indicated that $G_{\alpha q}$ and $G_{\alpha i}$ interact with the receptor in a sequential manner. The sequential receptor/G-protein interaction was correlated with the timing of the following signaling cascades, which might reflect a potential mechanism for pathway-specific signal regulation.

In **Chapter 4** a newly emerging approach of optogenetics was exploited to develop an all optically-controlled chimeric receptor optoCXCR4. A detailed description of the design of a chimeric receptor is present together with various experiments to test for proper functionality. The developed construct represents a promising tool for further biophysical investigation of chemokine receptor CXCR4 signaling, that permits external cellular control at high temporal and spatial resolution.

In **Chapter 5** a hypothesis of the mechanical guidance of Ewing sarcoma metastasis was examined. The influence of the mechanical properties of the microenvironment on ES was tested in 2D and 3D assays. Various Ewing sarcoma derived cells exhibited an evident difference in mechanical phenotype depending on their metastatic niche. The softer microenvironment appeared more attractive for cells derived from patients with lungs metastasis localisation, while stiffer substrates resulted

in higher adhesion potential for cells derived from patients bone metastasis localization. Notably, activation of the CXCR4 receptor had no evident effect on the mechanical phenotype.

1.8 Appendix

The history of Ewing sarcoma starts in 1920's, when the pathologist J. Ewing published his first work on a new kind of bone cancer.

James Ewing, born in 1866, graduated from Columbia University College of Physicians and Surgeons in 1891. In 1899 Ewing became the first professor of pathology at Cornell University Medical College (Huvos, 1998). In 1913 Ewing became the first Director of Pathology at present-day Memorial Sloan-Kettering Cancer Center in New York City (then called New York Cancer Hospital) (Huvos, 1998; www.mskcc.org). In 1931, after his retirement from Cornell as Chairman of the Department of Pathology, Ewing became the Director of Memorial Hospital for Cancer and Allied Diseases (Huvos, 1998).

At the time Ewing was one of the central figures in many aspects of cancer-related research and owned to be named "Cancer Man" by the Times Magazine (Fig. A1) (www.time.com). His work was considered as an excellent starting point for any aspect of oncology (Shimkin, 1974). Under J. Ewing guidance the New York Cancer Hospital became world-wide recognized in the diagnosis and management of neoplastic diseases (www.mskcc.org). He was a 'father' of the American Society of the Cancer Control (A.S.C.C., 1913) (Triolo and Shimkin, 1969), and President of the American Association for Cancer Research (AACR) in 1907-09 (Triolo and Riegel, 1961). As member of the AACR Ewing was involved in the establishment of Journal of Cancer Research (Cancer Research at present-day) in 1916, and its reformation into the American Journal of Cancer in 1930 (Triolo and Riegel, 1961).

In 1919 the first edition of Ewing book 'Neoplastic Diseases: A Text-Book on Tumors' was published (Ewing, 1922). The book gave a comprehensive overview and classification of the available studies on neoplastic diseases (Ewing, 1922; Huvos, 1998) and provided a systematic basis for diagnosing human cancer, representing a keystone of modern oncology (www.mskcc.org). Already in 1922 the second edition of the book was published. It included studies collected over the past two years, new microphotographs and a major correction on 'Tumor of bone' chapter (Ewing, 1922). In his book (and lectures) Ewing described a special case of a 14-year-old girl patient with a large tumor of the ulna (Ewing, 1922; Huvos, 1998). The most abundant treatment at the time was amputation. However, given his personal history of facing a potential leg amputation at age of 14, Ewing was not in favor of such solution for

Figure A1
'Cancer man'

his patient. A treatment with only x-ray irradiation was used instead. The treatment resulted in disappearance of the tumor (Huvos, 1998). Together with multiple similar cases, this observation and collaborative work with Dr. Douglas lead to foundation of a radium department in the New York Cancer Hospital in 1915, and thus gave birth to radiation therapy in the United States (www.mskcc.org; Huvos, 1998).

Ewing noticed, that radiosensitivity was shared by a specific type of bone sarcoma, characterized by the typical microscopic appearance and known as round cell sarcoma. He described his observations and the 14-year-old girl case in his first publication on a new kind of malignant osteoma - 'diffuse endothelioma of bone' in 1921 (Choudhury et al., 2011; Ewing, 1972; Huvos, 1998). This is were the origin of the long history of Ewing sarcoma takes its start.

By 1927 there were more case reports with symptoms similar to the 'new' bone cancer. Representing 7% of the total bone cancer reports at the time, it was appointed the name 'Ewing sarcoma' (Pritchard, 1927). Extensive research and development in cytogenetics resulted in the establishment of the common chromosomal translocation which became characteristic to Ewing sarcoma in 1980's (Fraccaro et al., 1980; et al.,

1983; Turc-Carel et al., 1988). Around 1987 the break points of reciprocal translocation at 11q24 and 22q12 were confirmed to be uniform for Ewing sarcoma. In 1994 the resulting fusion-protein was identified (Delattre et al., 1994). From then on the presence of the fusion-proteins EWS/FLI or EWS/ERG became a defining criterion for Ewing sarcoma (Delattre et al., 1994).

Today Ewing sarcoma is the second most common bone cancer in children and young adults. The average patients age is 13 years with a range from 1 to 48 years. It is described as small-round cell tumor and identified by molecular genetic analysis for the t(11,22)q(24;12) translocation and the expression of EWS/FLI or EWS-ERG. (Delattre et al., 1994; Dockhorn-Dworniczak et al., 1994; Ludwig, 2008; Sand et al., 2015).

Ewing worked through his life to better understand and search ways to fight the disease of cancer. He made a huge impact, particularly on the cancer of bone. Through almost 100 years Ewing sarcoma still holds the name of a great pathologist and oncologist of early 20th century James Ewing. Tragically himself Ewing succumbed to bladder carcinoma in 1943 (Huvos, 1998).

1.9 References

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