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Integrin signaling modes controlling cell migration and metastasis

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Summary and discussion

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Embryo development, wound healing, angiogenesis, and cancer metastasis rely on cell motility and adhesion dynamics. This involves shifting integrin expression profiles, which may reflect the changing environment that the cells encounter and adapt to. For example, malignant cells migrating from primary to secondary sites will come across extracellular matrix (ECM) compositions that are different from their site of origin. In ***chapters 2 and 3*** of this thesis, the roles of $\alpha5\beta1$ and $\alpha v\beta3$ fibronectin (FN)-binding integrins in cell motility and adhesion dynamics are discussed. We find that when these integrins are ectopically expressed in the same cellular background (GE11), striking differences in cellular functions (cell morphology, cell-matrix adhesion dynamics and localization) are observed. Cells expressing $\alpha5\beta1$ integrin exhibit a contractile fibroblastic morphology with highly dynamic centripetally orientated cell-matrix adhesions and migrate in random fashion. In contrast, $\alpha v\beta3$ expressing cells, whose cell-matrix adhesions are more static and distributed across the basal surface, migrate in a highly persistent fashion.

What is the reason for these differences when both integrins interact with FN? One possible explanation could be the interaction of signaling and/or adaptor proteins with specific residues within the integrin cytoplasmic tail. However, α- and β- tail swapping experiments revealed that this is probably not the case. Rather, our evidently suggests that it is how the integrins interact with the FN that affects cell signaling. We demonstrate that both $\alpha5\beta1$ and $\alpha v\beta3$ adhere to immobilized (stretched) FN, but only $\alpha5\beta1$ binds to soluble folded (inactive) FN. RGD in immobilized (stretched) FN is reoriented in such a way that it is accessible to both integrins (Altroff et al., 2004). The ability of $\alpha5\beta1$ to access the RGD site in soluble FN comes from the fact that this integrin has an additional FN binding region (Aota, Nomizu, and Yamada, 1994; Bowditch et al., 1994; Danen et al., 1995) and we show that placing the CTSEQNC hypervariable sequence in the I-like domain of $\beta1$ in the context of $\beta3$ allows $\alpha v\beta3$ to also bind soluble FN. It has been suggested that the $\alpha5\beta1$ hypervariable sequence binds to the PHSRN “synergy” region in IIIFN9 to stabilize interaction with the RGD region in IIIFN10 by changing the tilt angle between IIIFn10 and IIIFn9, subsequently exposing RGD loops. Our findings demonstrate that integrin $\alpha v\beta3$, even if locked in a high affinity state by different mutations, binds poorly to soluble FN because it lacks

this functionality (Danen et al., 1995; Sechler, Corbett, and schwarzbauer, 1997).

The association with the synergy region may explain the apparently specific ability of $\alpha5\beta1$ to form a "catch-bond" with FN and mediate adhesion strengthening (Friedland, Lee, and Boettiger, 2009; Roca-Cusachs et al., 2009). Importantly, our findings indicate that switching between these two integrins with such distinct ligand-interaction modes strongly affects intracellular cellular with effects on RhoA activity, cytoskeletal contractility, and ECM assembly.

In ***chapter 4***, a novel $\alpha v\beta3$ binding partner is described that could also contribute to the specific $\alpha v\beta3$-mediated effects on cell morphology. MacMarcks (MRP) had been implicated in the activation of integrins and cell spreading by regulating the cortical actin network (Jin and Li, 2002). Interestingly, expression of $\beta3$ integrin transcriptionally down-regulates MRP. We demonstrate that the region in the vicinity of NITY domain of the $\beta3$ tail down-regulates MRP if associated with the αv-subunit. Nonetheless, silencing MRP did not promote cell spreading in the parental line, and overexpression did not hamper cell spreading in cells using $\alpha v\beta3$ for adhesion. This suggests that $\beta3$ is required for MRP localization and expression but MRP is not essential for $\alpha v\beta3$-mediated cell spreading (in contrast to $\beta2$ integrins, which have been claimed to depend on MRP for spreading (Li et al., 1996).

As described in ***chapter 5***, certain diseases display altered expression or functionality of integrins. For instance, high expression levels of various types of integrins have been correlated with tumor progression in a numbers of cancers (Mizejewski, 1999). For that reason, antagonists such as peptidomimetics and monoclonal antibodies have been developed targeting either $\alpha5\beta1$ and $\alpha v\beta3$ integrins. Disintegrins are RGD-containing cysteine-rich peptides in snake venom that have been developed as therapeutic agents for angiogenesis-dependent tumor growth and metastasis (Huang, 1998). Unfortunately, these molecules are very large and have low metabolic stability limiting their use for clinical applications (McLane et al., 2004, Cai and Chen, 2006). Cyclic RGD-containing pentapeptides are the most commonly used RGD-based antagonist (Ruoslahti, 1996). c(RGDf(NMe)V) a.k.a Cilengitide (EMD 121974) (Goodman et al., 2002) has effectively induced apoptosis in glioblastoma and medullablastoma (Taga et al., 2002). Integrin antagonist can be applied in combination with cytotoxic

anticancer therapy, such as chemo- or radiotherapy to maximize therapeutic efficacy. For example, Cilengitide in combination with gemcitabine inhibits highly vascularized tumor growth (Colomer, 2004; Raguse et al., 2004). It has been reported that tumor-associated endothelial cells can evade death by up-regulating $\alpha v\beta 3$ integrin upon radiation exposure. Agents such as the $\alpha v\beta 3$ inhibitor S247 have the potential to block growth of tumor cells and angiogenic vessels and cause inhibition of phosphorylation of PKB/Akt (Abdollahi et al., 2005).

Classic 2D culture conditions differ strongly from the in vivo situation and affect cell survival, proliferation, differentiation, cytoarchitecture, and migration (Kenny PA et al., 2007; Bjerkvig, 1990; Bissell, 1981; Wapita and Hay, 2002; Corcoran et al., 2003; Beliveau et al., 2010). For cancer metastasis-related studies, 3D invasion assays such as the Boyden chamber assay (trans-well migration assay) also may not properly resemble tumor cells disassociating from a solid tumor. For this purpose, cell spheroid (CS) cultures have been developed that mimic solid cancer microenvironments. This requires CS to be compact and contain an oxygen- and nutrient-depleted core, which are characteristics of solid tumors (Mueller-Klieser, 1987; Sutherlands, 1988). In addition, the ECM environment surrounding CS ideally mimics chemical (ECM protein type) and physical (rigidity, cross-linking) properties of tissue (Buxboim and Discher,2010; Friedl and Wolf, 2010, Leventhal et al., 2009). In ***chapter 6***, we describe the development a novel CS formation method in 3D collagen gels that fulfills these criteria and, for the first time, can be performed in high throughput with high accuracy and reproducibility.

There are several advantages of our approach based on microinjection over other methods. For one, we combined CS formation and gel embedding into a single step, thereby shortening preparation time from days to minutes. Secondly, CS formation from a broad spectrum of cell can be achieved without additive, e.g. matrigel such as used by others (Ivascu and Manfred, 2006, 2007). Thirdly, CS are produced with uniform size and shape with predefined spatial distribution, making this method ideal for HTS. Unlike other techniques, 2D tissue culturing steps are completely omitted, and freshly isolated tumor samples of mouse and human biopsies can be used directly for CS formation. Consequently, we have designed an automated 3D culture syste

that may be applied to drug screens for personalized treatment strategies.

According to several studies, β1 integrins support initiation and growth of breast cancer. By blocking β1 integrins with antibodies, breast tumors in mice have been sensitized to radiotherapy, indicating that β1 integrins may be suitable drug targets for breast cancer (White et al., 2004; Park et al., 2006). In ***chapter 7***, we describe that silencing β1 in breast cancer cells indeed suppresses tumor growth but can also lead to enhanced intravasation and metastasis. Our data support a model where in the absence of β1 integrins, an epithelial-to-mesenchymal (EMT) transition is induced through transcriptional down regulating of E-cadherin by an altered balance between the ZEB and mir-200 families. Consequently, cells shift from cohesive multicellular strand invasion to individual cell migration in 3D matrices. This likely enables tumor cells to intravasate more efficiently through enhanced migration by eliminating the burden to travel as collective units (dragging force) or it may up-regulate survival mechanisms to resist the sheer stress within the blood vessel; or speed up.

Experiments using inhibitory peptides and α2 subunit silencing constructs, indicate that α2β1 is the β1 integrin that acts as a metastasis suppressor in this system. Interestingly, this integrin was very recently shown to be inversely correlated with breast cancer progression (Ramirez et al., 2010). Nevertheless, we do not detect a general loss of α2 or β1 integrins to correlate with E-cadherin loss. Histological samples provide a snapshot in the dynamic process of metastasis process and may not reveal key transient malignant modifications in the metastatic cascade; especially if these are rare and transient. Indeed, the down-regulation of E-cadherin in response to decreased β1-integrin-mediated adhesion may be such a transient process that occurs in a subpopulation of cancer cells within a tumor. Such events may be missed by the pathologist but play a role in metastasis of E-cadherin positive breast cancers.

From fundamental research to clinical application, integrins have presented themselves to be highly intriguing receptors. Besides mediating cell attachment to the microenvironment, integrins organize signal transduction cascades that regulate cell biology from proliferation and survival to migration. As such, they appear to be useful biomarkers and drug targets in multiple diseases, including cancer.

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