immobilized integrin receptor (i.e., $\alpha_5\beta_1$ and $\alpha v\beta_3$) and found that only echistatin inhibited fibronetin binding to $\alpha_5\beta_1$, and all disintegrins except barbourin (a specific $\alpha_{IIb}\beta_3$ blocker) blocked the vitronectin binding to $\alpha v\beta_3$.[45] Further studies indicate that the amino acid sequence immediately adjacent to the RGD site of disintegrin can create an extended RGD locus, which in coupling with the conformational effect on the RGD sequence may be involved in determining the affinity and selectivity toward integrins. A recent study with echistatin mutants suggested that the RGD loop and the C-terminus bind to different sites within integrins β3 and β1.[46] However, the precise interaction between disintegrin and each integrin on a molecular basis is still not fully elucidated.

In a number of studies, disintegrins have been shown to inhibit platelet aggregation induced by tumor cells.[46-48] It is thought that the inhibitory effect of disintegrins on tumor cell adhesion to ECM and tumor cell-induced platelet aggregation may be partially responsible for their in vivo antimetastatic activity.

Both echistatin and flavoridin inhibited in vitro retinal detachment, induced by retinal pigment cells attached to ECM, and consequent vitreous contraction.[49] Yang et al. showed that triflavin inhibited retinal pigment cell adhesion to ECM. Triflavin exerted a potent effect in inhibiting rat mesangial cell adhesion to fibronectin and collagen types I and III, and inhibited platelet-derived growth factor (PDGF)-promoted mesangial cell growth in serum-free medium.[50] Sheu et al. demonstrated that triflavin may inhibit aggregating platelet-induced vasoconstriction in de-endothelialized rat aorta.[51] Echistatin has been used to isolate pure cultures of mammalian osteoclasts.[52] At low concentrations, it has also prevented bone destruction by interacting with $\alpha v\beta_3$ expressed on osteoclasts.[53] $\alpha v\beta_3$ is needed in smooth muscle cell migration mediated by insulin-growth factor-1 stimulation or by PDGF; and kistrin, echistatin, or echispydin modulated this process.[54] By using the recombinant rhodostomin as a substrate, osteoprogenitor-like cells can be selected. Rhodortomin can be immobilized on beads which serve as an affinity column to dissect cell-surface protein(s) bound to the RGD motif of rhodostomin.[98]

Disintegrins and Angiogenesis

Integrin $\alpha v\beta_3$ expressed on many cells (including vascular endothelial cells, smooth muscle cells, and fibroblasts) modulates cell migration and cell proliferation and has an impact on angiogenesis, restenosis, tumor cell invasion, and atherosclerosis.[55] The ligands include fibrinogen, fibrin, fibronectin, vWF, osteopontin, and vitronectin.[6] Because they are RGD-containing adhesive proteins, RGD-containing peptides inhibit binding of these ligands to $\alpha v\beta_3$. Angiogenesis plays an important modulatory role in normal physiological processes, such as embryonic development, tissue repair, and luteal formation.[56] On the other hand, angiogenesis is intimately involved in some pathological conditions, such as promoting tumor growth and eliciting diabetic retinopathy and inflammatory diseases (e.g., rheumatoid arthritis).[57] The involvement of $\alpha v\beta_3$ in angiogenesis was first demonstrated by Cheresh et al.[58,59] In the chick choriallantoic membrane (CAM) model, LM609, an anti-$\alpha v\beta_3$ monoclonal antibody, inhibited neovascularization induced by implanting an $\alpha v\beta_3$-negative melanoma or by implanting a basic fibroblast (b-FGF) factor-containing pellet on the CAMs of 10-day-old embryos. The cyclic RGD derivative inhibited tumor-induced angiogenesis and hypoxia-induced neovascularization in the murine retina.[60] LM609 currently is under clinical trial for preventing tumor metastasis.

As mentioned above, disintegrins inhibit adhesion between tumor cells, HUVECs, and ECM through blockade of the integrins $\alpha v\beta_3$ and $\alpha_5\beta_1$. We previously reported that rhodostomin inhibited prostaglandin I_2 formation of HUVECs caused by ancrod (a thrombin-like venom protein)-generated fibrin through an $\alpha v\beta_3$ blockade.[61-63] Disintegrins (e.g., accutin, rhodostomin, triflavin, and salmosin) dose-dependently display inhibitory activity on cell adhesion to ECM, cell proliferation, matrigel-induced capillary tube formation, and neovascularization of the CAM model, mainly through the blockade of HUVEC $\alpha v\beta_3$.[64,65] Furthermore, rhodostomin was found to inhibit neovascularization induced by either B16F10 tumors or b-FGF, but not by vascular endothelial growth factor (VEGF), through an $\alpha v\beta_3$-dependent mechanism. In