

Podosomes and Invadopodia Help Mobile Cells Step Lively

These feetlike structures aid the necessary migrations of immune and other cells, but also the deadly wanderings of cancer cells

Anyone who has taken an introductory biology class is familiar with the migratory prowess of the simple amoeba. But many of our own cells could give amoebae a run for their money. Immune cells have to sprint to infection sites to ward off invading pathogens. And cells that help maintain the skeleton nimbly patrol bones. On the minus side, however, cancer cells can travel throughout the body and seed new tumors.

Recently, a couple of hitherto-obscure cellular structures, known as podosomes and invadopodia, have come under increasing scrutiny as possible contributors to such perambulations. These microscopic assemblies, which are possibly related, both form on the bottom surface of the leading edge of migrating cells—a location consistent with the idea that they help cells move. The composition of each reinforces that suspicion: Both contain proteins, such as actin, that have previously been linked to cell motility (*Science*, 10 October 2003, p. 214), as well as enzymes that can break down the proteins of the extracellular matrix (ECM).

Establishing the roles of podosomes and invadopodia hasn't been easy, however, because they have so far been studied in cells maintained on artificial surfaces rather than in live animals. "As far as I know, no one has seen these structures in vivo," notes Gareth Jones of King's College London.

Still, recent research suggests that these cellular features are crucial for understanding and perhaps treating a variety of diseases as diverse as cancer and osteoporosis. Indeed, scientists have already implicated malfunctioning podosomes as a cause of a rare hereditary disease that impairs the immune system. Research on podosomes and invadopodia "is heating up because they've really become physiologically important for things such as metastasis," says cell biologist John Condeelis of Albert Einstein College of Medicine in New York City.

Feet for cells

Podosomes first appeared on cell biologists' radar screens in the mid-1980s. Pier Carlo Marchisio, currently at San Raffaele Scientific Institute in Milan, Italy, and his colleagues discovered the structures on a variety of migratory cells, including immune cells called macrophages and osteoclasts, which help maintain bone by dissolving away areas that need repair. In all cases, the structures appeared where the cells made contact with a surface. This suggested that they might be involved in cell adhesion, and thus in cell motility,

because cells have to stick to the surface over which they are migrating in order to move. Because the structures appeared to act like cellular feet, the Marchisio team coined the name podosomes for them.

Invadopodia came along just a few years later, identified in 1989 by Wen-Tien Chen, now at Stony Brook University in New York. He found that cells transformed by Rous sarcoma virus (RSV) form protrusions at their leading edge when moving on a surface containing ECM proteins such as fibronectin. More intriguing, Chen noticed that holes appeared in the protein substrate at the precise spots where the protrusions were located—an indication that they carry proteases that digest the ECM.

"Invadopodia are more than just feet," Chen says. "They have a functional effect." The ability to digest ECM proteins would be very useful to migrating cells, including cancer cells that need to burrow through blood vessel walls in order to spread to distant sites.

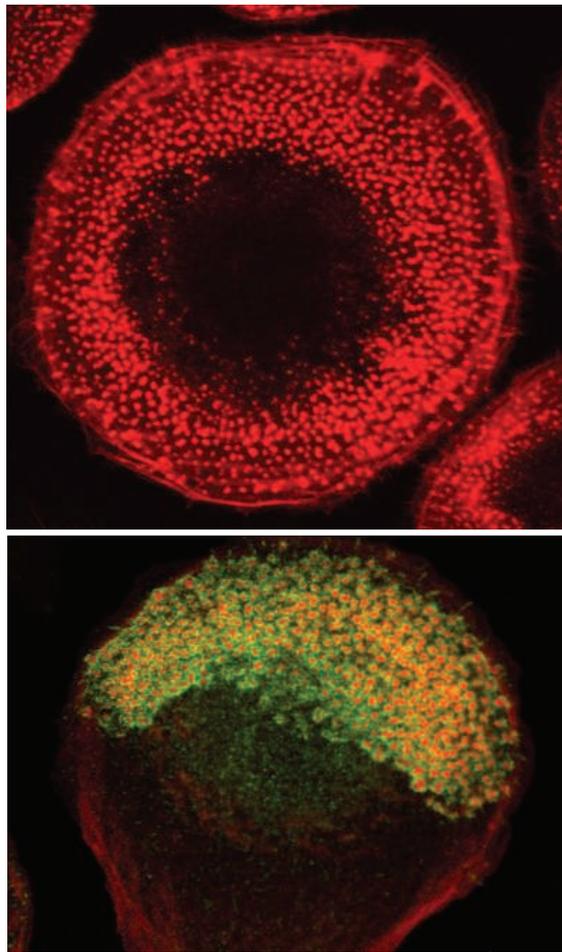
Marchisio and his colleagues found similar structures in cells transformed by RSV but considered them to be podosomes. Both researchers now say that they think they were looking at the same thing, yet the precise relationship between podosomes and invadopodia remains a burning issue in the field. Most researchers today define podosomes as more dynamic than invadopodia and smaller—1 to 2 micrometers in diameter compared to 8 to 10 micrometers. But both structures have a core of actin filaments and contain other proteins that regulate actin polymerization. Some researchers, including Roberto Buccione of the Consorzio Mario Negri Sud in Chieti, Italy, and Mark McNiven of the Mayo Clinic in Rochester, Minnesota, have suggested that podosomes form first and then mature into invadopodia, but the jury is still out on this idea.

The buzz about WASp

Research on podosomes took off in the late 1990s when they were linked to the then-newly-discovered mutant gene that causes Wiskott-Aldrich syndrome (WAS). Because the gene is on the X chromosome, the disease afflicts mainly boys, causing them to be severely immunodeficient. Without a bone marrow transplant, the children usually die before age 20 of infections or of immune-cell cancers to which they are also prone.

Early analysis of WASp, the protein product of the gene that was found to be mutated in the immune syndrome, suggested that it helps control actin function. Then in the late 1990s, Stefan Linder of Ludwig Maximilians University in Munich, Germany, and his colleagues detected WASp in the actin core of macrophage podosomes. Linder's group and another team led by Jones and Adrian Thrasher of University College London also found evidence that podosome formation requires WASp. "Normal macrophages and dendritic cells have these structures, but cells from boys with Wiskott-Aldrich had none at all. That was a bit of a shock," Jones recalls.

The London team found that the patients' macrophages had also lost the ability to respond to chemokines and cytokines, the chemical signals that normally activate immune cells and attract them to infection sites—a possible explanation for the



Going mobile. In a moving macrophage (*bottom*), podosomes (stained red for actin and green for vinculin) congregate on the cell's leading edge while they ring the bottom of a quiescent macrophage (*top*, stained red for actin).

impaired immunity of WAS patients. Both podosome formation and the ability to migrate in response to chemical signals were restored by putting the normal *WAS* gene into patient macrophages. “This was the first link [of WASp] to something physiological,” Linder says.

Further evidence for a physiological link came from a study that Jones, Thrasher, and their colleagues described in *Blood* in February of last year. Dendritic cells serve as sentinels throughout the body, detecting foreign invaders and initiating immune responses to them. The London team found that the dendritic cells of mice in which the *WAS* gene had been deactivated could not migrate to their normal locations in the lymph nodes, presumably because they can’t form podosomes.

Cancer connection

The ability to form podosomes and respond to chemokines could have a dark side: Cancer cells may exploit those same skills to spread. In recent work, Condeelis and his colleagues have been teasing out the changes in gene expression that characterize metastatic cancer cells. Having previously shown that the metastatic potential of cancer cells correlates with the cells’ ability to migrate toward epidermal growth factor (EGF), the researchers inserted tiny needles containing EGF into mammary tumors in rodents and then analyzed the gene expression patterns of the cells that moved into the needles. These cells had increased expression of numerous genes that promote motility and podosome/invadopodia formation, including WASp. Activation of the genes “determines the willingness and ability [of cancer cells] to move to a portal to escape the tumor,” Condeelis says.

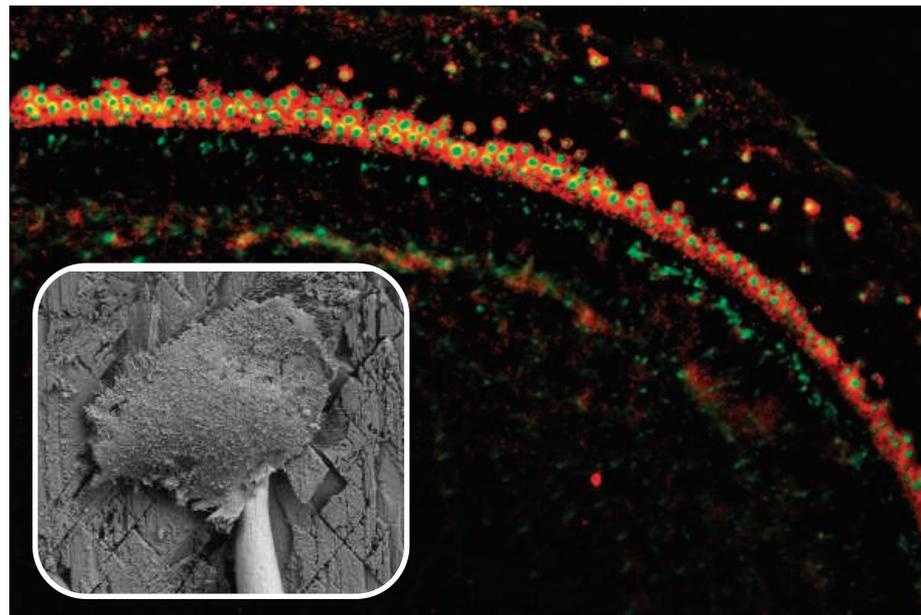
Other work by the Albert Einstein team indicates that macrophages and their podosomes are partners in the crime of metastasis. The researchers find that as mammary cancer cells migrate into the blood vessels, they move along with the immune cells. The two cell types pair up because each produces a growth factor that attracts the other: Macrophages associated with the blood vessels attract metastatic cancer cells by secreting EGF, and the cancer cells emit a protein called CSF-1 that attracts more macrophages and stimulates further EGF secretion by the immune cells.

The CSF-1 made by cancer cells also induces in macrophages the expression of WASp and other genes involved in podosome formation; similarly, macrophage-made EGF fosters invadopodia formation by the cancer cells. Macrophages could further help tumor cells penetrate the blood vessel wall; recent work by the Condeelis team has shown that the immune cells’ podosomes, like invadopodia, carry enzymes that can digest the ECM. Chen suggests that inhibiting such enzymes, particularly one called seprase that his team has found in the invadopodia of melanoma cells, may thwart metastasis.

In the 1 May issue of *Cancer Research*, Chen’s group reports that seprase and a similar enzyme, DPP4, are also located in a complex on invadopodialike structures in endothelial cells. Because tumor growth depends on the creation of new blood vessels by migrating endothelial cells, that finding hints at yet another anticancer strategy: Chen’s team

results, with some suggesting that the two structures are independent of each other.

Jurdic, Destaing, and their colleagues have found, however, that sealing belts only form when osteoclasts are in contact with apatite, the mineral that forms the solid framework of bone. Apatite somehow activates both the Rho and Src kinase enzymes, both of which are needed for



Bone repair. In an osteoclast, podosomes (stained red for paxillin and green for actin) form a belt on the cell periphery. The podosome belt may form the sealing ring that attaches an osteoclast (*inset*) to a bone mineral site that needs repair.

blocked endothelial cell migration in lab dishes with monoclonal antibodies targeting the seprase-DPP4 complex.

Boning up on podosomes

The osteoclasts of bone are also migratory cells that may depend on podosomes for their mobility—and perhaps for much more. The cells seek out those areas of bone that need repair. Once there, an osteoclast settles down and forms a structure called a sealing ring, a tight connection between cell and bone. This protects adjacent bone from the osteoclasts. “For an osteoclast to be functional, it needs to isolate its target from the rest of bone; it secretes lots of concentrated acid,” says Olivier Destaing of Yale University School of Medicine.

Podosomes appear to be involved in sealing-ring formation. Pierre Jurdic of the École Normale Supérieure de Lyon in France and his colleagues, including Destaing, found that as osteoclasts develop, they first produce individual podosomes that arrange themselves in small rings. These rings then expand to the periphery of the cell, where they form a stable podosome belt. The early view was that the podosome belts might coalesce to form sealing rings, which are thicker, but efforts to detect that transition had produced contradictory

sealing-ring formation. The researchers also found that inhibiting Rho induces the transition of sealing rings into podosome belts.

Further support for the idea that the structures are related comes from Benjamin Geiger’s group at the Weizmann Institute of Science in Rehovot, Israel. His team found rearrangements in podosome proteins—in particular, a marked increase in their levels—at locations where sealing-zone structures appeared to be forming from podosome rings. This would be consistent with the notion that sealing rings are thicker versions of podosome belts. If so, drugs that inhibit podosome function in osteoclasts could form the basis of a new osteoporosis therapy; the disorder typically results when the bone-dissolving activities of osteoclasts outpace the bone-forming capacities of their partners, the osteoblasts.

Many questions about podosomes and invadopodia remain to be answered. In particular, researchers are searching for markers that would let them observe the structures in living tissue—and perhaps put to rest the nagging doubts about the relevance of lab-dish studies. That would certainly give them a step up on understanding these cellular feet that are now attracting so much interest.

—JEAN MARX