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REVIEW ARTICLE

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The role of c-Src kinase in the regulation of osteoclast function

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Abstract The targeted disruption of c-Src impairs osteoclast bone resorbing activity, causing osteopetrosis. Although it has been reported that restoring only the c-Src adaptor function at least partly rescues the skeletal phenotypes, the importance of c-Src kinase activity remains controversial. We here highlight the contributions of the Src adaptor and kinase activities in cytoskeletal organization and osteoclast function using adenovirus vectors containing various mutants of Src or Pyk2. In addition, we describe the importance of c-Src in mitochondria, where it phosphorylates cytochrome *c* oxidase (Cox). Src-induced Cox activity is also required for bone resorbing activity of osteoclasts that require high levels of ATP. Thus, c-Src kinase activity not only on the plasma membrane but also within mitochondria is essential for the regulation of osteoclastic bone resorption.

Key words c-Src · Kinase · Mitochondria · Osteoclast · Pyk2

Introduction

Non-receptor type tyrosine kinase c-Src is a member of a family of nine protein–tyrosine kinases that associate with the cytoplasmic surface of the cellular membrane.[1] Activated mutants of Src are oncogenic, and study of these mutants has implicated c-Src in the control of cell growth and proliferation. However, c-Src is highly expressed in terminally differentiated cells such as platelets and neurons, indicating a physiological role for the protein unrelated to growth control.[2] Soriano et al.[3] reported that targeted disruption of the c-*src* gene in mice induced osteopetrosis, a disorder characterized by decreased bone resorption, without showing any obvious morphological or functional abnormalities in other tissues or cells. They also showed that the osteopetrotic phenotype of c-*src*-disrupted mice is cell-autonomous and occurs in mature osteoclasts.

Osteoclasts are multinucleated, terminally differentiated cells which degrade mineralized matrix during normal and pathological bone turnover.[4] Osteoclastic bone resorption involves the proliferation and homing of the hemopoietic osteoclast progenitors to bone, their differentiation and fusion to form multinucleated cells, and the migration of osteoclasts to and between the resorption sites. Osteoclasts attach to the bone surface and form a tight sealing zone (or "clear zone") enclosing the resorption lacunae, which was frequently compared to a large lysosome. Following the insertion of secretory vesicles, a highly convoluted membrane called the "ruffled border" is formed facing the bone surface.[5] Integrins have been suggested to play a role in osteoclast activity by mediating osteoclast adhesion and regulating the cytoskeletal organization required for both cell migration and formation of sealing zone. v-*src*-transformed cells contain high concentrations of Src as well as other tyrosine phosphorylated proteins in podosome,[6,7] specialized adhesion sites that are structurally and functionally distinct from focal adhesions.[6,8–10] Although many of the same proteins are present in podosomes and focal adhesions, focal adhesions are relatively stable, whereas podosomes are dynamic attachment structures, undergoing assembly and disassembly within minutes.[11–13] Activation of Src may therefore be associated with a shift from stable focal adhesions with actin stress fibers to more dynamic podosome assemblies, possibly regulating cell motility. Indeed, several papers have suggested a role for Src kinase activity in cell spreading and migration.[14–18]

It remains uncertain, however, whether catalytically active Src is required for normal osteoclast function.

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Schwartzberg et al.[19] previously reported that osteoclast-specific expression of kinase-dead Src mutants rescued the $Src^{-/-}$ osteopetrotic phenotype, suggesting that c-Src may act as an adaptor molecule and that c-Src kinase activity may not be critical for bone resorption.[19] In addition, no adverse effects of kinase-dead Src expression in $Src^{+/+}$ and $Src^{+/-}$ animals was observed.[19,20] On the other hand, we have recently reported that down- or up-regulation of c-Src activity modulates osteoclastic bone resorption not only in vitro but also in vivo,[18] leaving the question of the contribution of c-Src kinase activity unsettled.

Here, we show that not only adaptor function but also the kinase activity of c-Src are important in osteoclastic bone resorption, taking advantage of the ability of adenoviral vectors to transduce foreign genes into osteoclasts.[21] In addition, we also found that c-Src in mitochondria regulates osteoclast function via cytochrome c oxidase activity.[22] In this review, we would like to introduce the role of c-Src kinase activity in both plasma membrane and mitochondria.

The structure of c-Src

Src family tyrosine kinases have a common domain organization, with each segment designated as a Src-homology (SH) region. The N-terminal segments includes the SH4 domain, which is a myristoylation and membrane-localization signal, as well as a "unique" domain, which differs among family members. This region is followed in the peptide chain by the SH3 domain, the SH2 domain, the tyrosine kinase (SH1) domain, and a short C-terminal tail, which includes a critical tyrosine residue (Fig. 1A). SH2 and SH3 domains mediate protein–protein interactions in cellular signaling cascades, and are found in many proteins outside the Src family. The SH3 and SH2 domains and the C-terminal tail all have roles in regulating Src kinase activity. It is now clear that phosphorylation of Tyr-527 by a specific kinase, Csk (C-terminal Src family kinase),[23] inhibits Src catalytic activity by creating an intramolecular binding site for the Src SH2 domain. The interaction is believed to result in auto-inhibition by locking the molecule in an inactive state. Displacement of SH2 domains by C-terminal tail dephosphorylation or by competitive binding of optimal SH2/SH3 domain ligands, allows the kinase domain to open, exposing Tyr-416 to phosphorylation. In open state, phosphorylation of Tyr-416 in the activation loop of the kinase domain further upregulates the enzyme (Fig. 1B).[24,25]

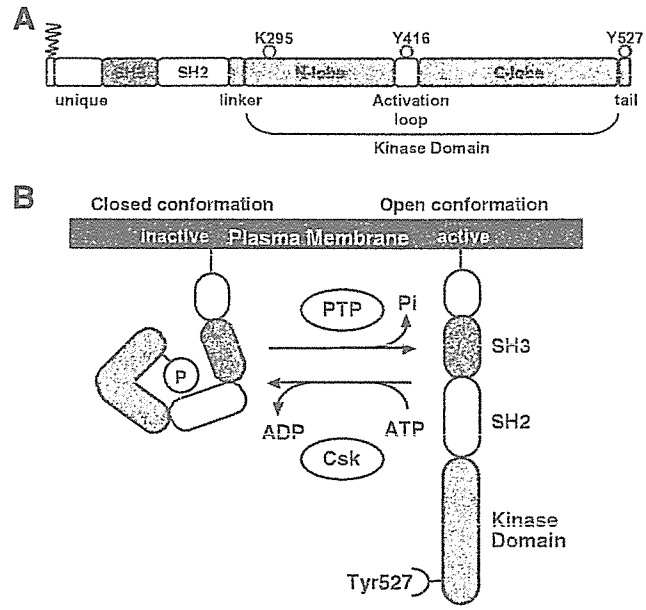

Fig. 1A,B. The structure of c-Src. **A** Schematic illustrations of the c-Src. N-terminal segment includes membrane-localization signal, myristoylation site (also called SH4 domain). The Src homology 3 domain, proline-rich binding site, SH2 domain, which binds to phosphotyrosine, and kinase domain follow in order. There are also a short, C-terminal tail, which includes a critical tyrosine residue. **B** The restrained conformation of c-Src is stabilized by intramolecular interactions among the kinase domain, the SH2/SH3 domains, and the phosphorylated C-terminal tail. Displacement of SH2 and/or SH3 domains, either by C-terminal tail dephosphorylation or by competitive binding of optimal SH2/SH3 domain ligands, allows the kinase domain to open, exposing Tyr-416 to phosphorylation

Pyk2-dependent recruitment of c-Src to the plasma membrane is necessary for bone resorption

Proline-rich tyrosine kinase 2 (Pyk2) has been identified as a major adhesion-dependent tyrosine kinase in osteoclasts, both in vivo and in vitro.[26–28] Pyk2 is a member of the focal adhesion kinase family, highly expressed in cell of the central nervous system and cells of hematopoietic lineage. Pyk2 and FAK share approximately 45% of the overall amino acid identity and have a high degree of sequence conservation surrounding binding site of SH2- and SH3-domain-containing proteins.[29] Ligation of $\alpha_v\beta_3$ integrin either by ligand binding or by antibody-mediated clustering increased Pyk2 tyrosine phosphorylation in osteoclasts. Moreover, in adherent osteoclasts, Pyk2 is tightly associated with c-Src, via its SH2 domain.[17,27] Upon osteoclast adhesion, Pyk2 translocates into the Triton X-100 insoluble cytoskeletal fraction and was concentrated at the cell periphery, co-localizing with F-actin.[27] Furthermore, Sanjay et al.[27] reported that Pyk2 is autophosphorylated upon integrin activation and that Pyk2 autophosphorylation at Y402 is required for the Pyk2/c-Src complex formation via the interaction with the Src SH2 domain. To examine the importance of the binding of Src to Pyk2, we constructed adenovirus vector carrying autophosphorylation site-mutated Pyk2 [$Pyk2^{Y402F}$]. More than 90% of wild-type osteoclast-like cells (OCLs) formed in vitro[30,31] display typical rounded appearances with a clear actin ring formation (Fig. 2A, upper panel). Adenovirus-mediated $Pyk2^{Y402F}$-overexpressed OCLs did not exhibit one large actin ring (Fig. 2A, lower panel) and strongly inhibited the bone-resorbing activity in proportion to $Pyk2^{Y402F}$ expression (Fig. 2B).[21] These data lead us to speculate that the interaction of

Src with Pyk2, mediated by the SrcSH2 domain and the Pyk2 Tyr(P)-402, is required for the normal organization of the osteoclast actin cytoskeleton and for bone resorption. Although the recruitment of Src to autophosphorylated Pyk2 at adhesion sites is a key event in bone resorption, it does not address the question of whether Src kinase activity is required or if the adaptor functions of Src SH2 and SH3 domains are sufficient. To examine whether or not the upregulation of Src kinase activity is sufficient to rescue the decreased pit-forming activity of $Pyk2^{Y402F}$-expressing OCLs, we coinfected OCLs with $AxPyk2^{Y402F}$ and adenovirus carrying kinase-dead C-terminal Src family kinase ($AxCsk^{KD}$), which prevents the phosphorylation of the negative regulatory Tyr-527 and increases Src kinase activity in OCLs.[18] The inhibitory effect of $AxPyk2^{Y402F}$ on osteoclast function was, however, not rescued by co-infection with $AxCsk^{KD}$, leading us to conclude that high Src kinase activity alone is not sufficient to rescue the decreased bone resorbing activity in the $Pyk2^{Y402F}$-expressing OCLs and that the Pyk2-dependent recruitment of Src is necessary for bone resorption.

c-Src kinase activity is required for osteoclast function

To further confirm the importance of Src kinase activity in osteoclasts, we constructed an adenovirus carrying kinase dead Src (Src^{K295M}). In contrast to the report by Schwartzberg et al.,[19] Src^{K295M}-expression completely disrupted the actin cytoskeleton in osteoclasts and bone-resorbing activity was strongly inhibited in proportion to the expression level of Src^{K295M} protein.[21] These results strongly suggest that it is the presence of the kinase activity of Src in the osteoclast adhesion structures and not just the Src protein that is necessary for actin ring formation and bone resorption. However, the autophosphorylation of Src promotes the adoption of the fully open active conformation,[24,25] and thus, Src^{K295M} might not be an effective adaptor.

To rule out the possibility that the dominant negative effects of Src^{K295M} could be due to a loss of adaptor function, we constructed $AxSrc^{K295M+Y527F}$, which prevents the phosphorylation of the negative regulatory Tyr-527 and promotes the availability of the SH2 and SH3 domains to bind other proteins. The morphology and bone-resorbing activity of $Src^{K295M+Y527F}$-expressing osteoclasts was similar to that of osteoclasts infected with $AxSrc^{K295M}$, further establishing that it is the absence of kinase activity, not the possible failure to bind to Pyk2 and/or other proteins in the adhesion complex, that causes the loss of bone resorbing activity.[21] To further confirm the importance of Src kinase activity, we investigated the functional changes in osteoclasts infected with $AxSrc^{Y416F+Y527F}$. The Y416F mutation reduces kinase activity about 50% by preventing the phosphorylation of a tyrosine on the activation loop of Src.[32,33] Despite its open conformation, $Src^{Y416F+Y527F}$ partially inhibited osteoclast function, consistent with its reduced kinase activity. $AxSrc^{Y416F+Y527F}$-infected osteoclasts exhibited a few small actin rings and still retained partial bone-resorbing activity (about 70% of control).[21] These results lead us to conclude that the localization of catalytically active Src in adhesion structures is required for actin ring formation and bone resorption, at least in vitro.

c-Src kinase activity is required for the efficient rescue of the c-$Src^{-/-}$ osteoclast phenotype

As noted earlier, Schwartzberg et al.[19] previously reported that osteoclast-specific expression of the kinase-dead Src^{K295M} mutant partially restored normal trabecular bone volume and tooth eruption in the $Src^{-/-}$ mice. We then introduced c-Src mutants into c-$Src^{-/-}$ osteoclasts to determine the influence of endogenous c-Src levels in our previous experiments. Under these in vitro experimental conditions, $Src^{-/-}$ OCLs were completely unable to form resorption pits in our assay (Fig. 4). Interestingly, Src^{K295M} and $Src^{K295M+Y527F}$, which did not notably affect the cytoskeletal organization of OCLs, restored (Fig. 3A), albeit extremely minimally (less than 3%), some bone-resorbing activity in $Src^{-/-}$ osteoclasts (Fig. 3B).[21] This minimal effect could be due to the adaptor function of c-Src or to the very low residual kinase activity in these mutants. In contrast, the expression of $Src^{Y416F+Y527F}$, which retains about 50% kinase activity, induced the formation of actin rings and very significantly increased bone-resorbing activity to ~70% of control (Fig. 3).[21] These results clearly show that c-Src kinase activity is indeed required to rescue the disruptive effect of c-Src deficiency on the cytoskeletal organization and bone-resorbing activity of osteoclasts.

The data from our study clearly show that at the level of the individual osteoclast, the kinase activity of Src is indeed required for bone resorption. Most interestingly, although $Src^{-/-}$ OCLs were 100% devoid of resorbing activity, cells reconstituted with Src^{K295M} demonstrated a very minimal (about 3%) but significant ($P < 0.001$) level of pit-forming activity (Fig. 3B), which was still much less than the >70% restoration of pit formation by OCLs reconstituted with $Src^{Y416F+Y527F}$. This minimal restoration of activity by Src^{K295M} suggests a possible explanation of the apparent discrepancy between our results with individual OCLs and the findings of Schwartzberg in animals. It is known that there are severalfold more osteoclasts in $Src^{-/-}$ than in wild type animals, and the degree of osteopetrosis in these animals is moderate.[34] Even a small increase in the activity of the larger number of osteoclasts acting over a longer time than our in vitro bone resorbing assay could induce sufficient additional bone resorption to account for the partial rescue reported by Schwartzberg et al.[19]

c-Src is localized in mitochondria

To define additional candidate proteins implicated in c-Src signaling, we performed a two-hybrid screen using kinase-

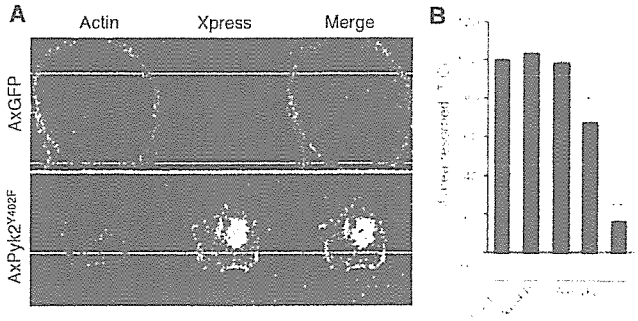

Fig. 2. **A** Double immunofluorescence staining of F-actin (*red*) and Xpress-tagged Pyk2^{Y402F} (*green*) in osteoclast-like cells (OCLs) infected with AxGFP or AxPyk2^{Y402F}. Adenovirus-mediated Pyk2^{Y402F} overexpression disrupted the one large actin ring formation. **B** Dentine-resorbing activity of OCLs expressing the Pyk2 mutants. OCLs 24 h after the infection at an indicated multiplicity of infection (*MOI*; a measure of titer, which reflects how many viruses infected a cell) were restored by digesting the collagen gel with 0.2% collagenase for 20 min at 37°C and an aliquot of the crude OCL preparation was transferred onto dentine slices (diameter 5 mm) as well as on 96-well culture plates and further cultured for 12 h. The resorbed area on dentine slices was measured using an image analysis system, and the number of TRAP-positive OCLs on culture plates was counted by light microscopy. The bone-resorbing activity of the cells was expressed as the resorbed area per osteoclast. The values are the mean ± SD ($n = 8$; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with non-infected OCLs)

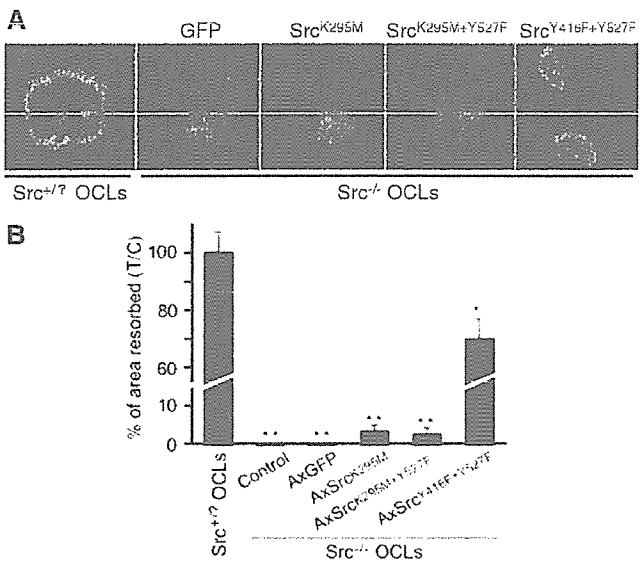

Fig. 3. **A** F-actin staining in Src$^{-/-}$ OCLs infected with AxGFP, AxSrc^{K295M}, AxSrc$^{K295M+Y527F}$, or AxSrc$^{Y416F+Y527F}$. Although Src^{K295M} and Src$^{K295M+Y527F}$ did not notably affect the cytoskeletal organization of Src$^{-/-}$ OCLs, the expression of Src$^{Y416F+Y527F}$ partly rescued the formation of actin rings. **B** Dentine-resorbing activity of Src$^{-/-}$ OCLs expressing the Src mutants. In contrast to Src^{K295M} and Src$^{K295M+Y527F}$, the expression of Src$^{Y416F+Y527F}$, which retains about 50% kinase activity, very significantly increased bone-resorbing activity. The values are the mean ± SD ($n > 8$; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with Src$^{-/-}$ OCLs)

dead c-Src as a bait. We screened a mouse brain library and obtained several clones that encode a mitochondrial protein. Mitochondria, the cellular energy plants, generate ATP through oxidative phosphorylation (OXPHOS). OXPHOS, defined as the oxidation of fuel molecules by

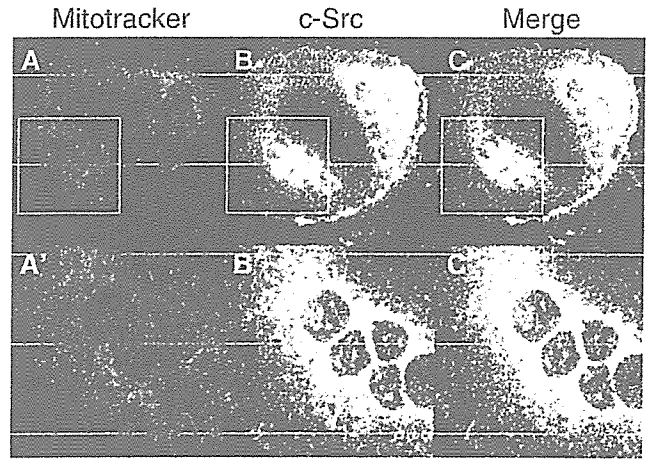

Fig. 4. Mitochondrial localization of c-Src. **A–C** Double immunofluorescence staining of Mitotracker and c-Src in osteoclasts. The localizations of Mitotracker (*red*) and c-Src (*green*) were assessed by confocal microscopy. **A′–C′** Close-up of the boxed region from **A**–**C**. c-Src staining was detected throughout the cytoplasm as well as at the cell periphery and many of these colocalized with mitochondria in osteoclasts

oxygen and the concomitant transduction of this energy into ATP, is the final process of the complicated biochemical network involved in cellular energy production. The OXPHOS molecular system, which is embedded in the lipid bilayer of the mitochondrial inner membrane, consists of electron acceptors, coenzyme Q and cytochrome c, and five multisubunit protein complexes (complexes I–V). The OXPHOS system comprises about 70 nuclear gene products and 13 mitochondrial gene products.[35,36]

To address whether c-Src might be present in mitochondria, we performed the dual staining of osteoclasts for c-Src and mitochondria using c-Src antibody and Mitotracker. As shown in Fig. 4, the merged image of c-Src (green) and mitochondria (red) revealed that c-Src protein appeared to colocalize with mitochondria. In close inspection of magnified images, c-Src clearly exhibited mitochondrial localization, as evidenced by colocalization with Mitotracker. The immunofluorescent results were also verified by immunoelectron microscopy. c-Src was found to be associated with the inner mitochondrial membrane in Src$^{-/-}$ osteoclasts. A large fraction of the gold particles were close to, or superimposed on, the inner mitochondrial membrane (Fig. 5). In contrast, no mitochondria showed labeling in the c-Src$^{-/-}$ osteoclasts (negative control).

We then examined the tyrosine phosphorylation of mitochondrial proteins from c-Src-overexpressing HEK 293 cells to determine if c-Src functions in mitochondria. Western blots of proteins suspended on two-dimension non-denaturing/denaturing gels with anti-phosphotyrosine suggested that one of the tyrosine-phosphorylated mitochondrial proteins was the cytochrome *c* oxidase (Cox).[22] Cox, which contains 13 subunits, is the terminal oxidase of cell respiration. The three major subunits of Cox are encoded by mitochondrial DNA and form the functional core of the enzyme; this core is surrounded by 10 nuclear-coded small subunits. Cox reduces dioxygen to water with four

Fig. 5. Immunogold labeling of c-Src in isolated mitochondria from c-Src$^{+/+}$ and c-Src$^{-/-}$ osteoclasts. As predicted from confocal microscopy data, there is strong labeling of mitochondria. Many of gold particles are associated with the inner mitochondrial membrane in c-Src$^{+/+}$ osteoclasts, whereas no labeling was detected in the mitochondria of c-Src$^{-/-}$ osteoclasts

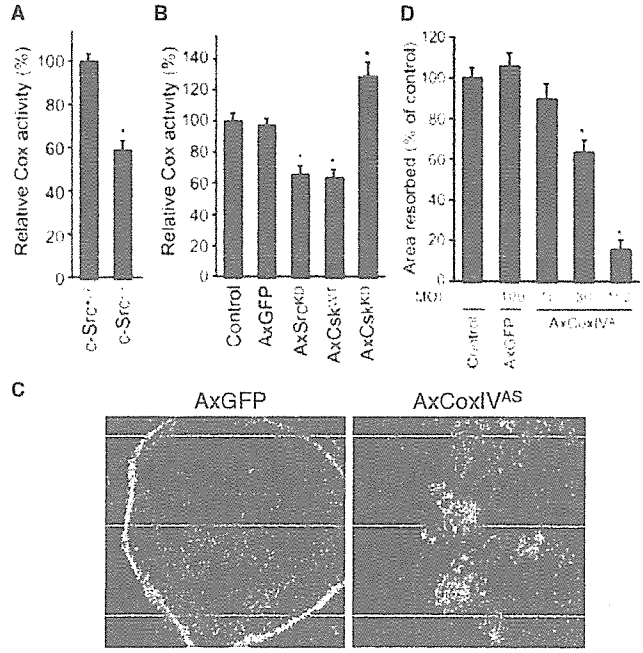

Fig. 6. **A** Cox activity in c-Src and c-Src$^{-/-}$ OCLs. Cox activity was reduced in c-Src$^{-/-}$ OCLs formed in vitro. The values are mean ± SD (n = 8; $^*P < 0.01$ compared with Src$^{+/+}$ OCLs). **B** Cox activity in OCLs infected with AxGFP, AxSrc^{KD}, AxCsk^{WT}, or AxCsk^{KD} at an MOI of 100. The values are mean ± SD (n = 8; $^*P < 0.01$ compared with uninfected OCLs). **C** Effects of CoxIV antisense (CoxIV^{AS}) on osteoclast morphology. Cocultures infected with AxGFP or AxCoxIV^{AS} at an MOI of 100 were plated on serum-coated glass coverslips for 12 h and then costained for F-actin using rhodamine phalloidin. **D** Dentine-resorbing activity of OCLs expressing CoxIV antisense. An aliquot of the OCL preparation was transferred to dentine and cultured for an additional 12 h. Bone-resorbing activity was progressively and severely inhibited as the MOI of AxCoxIV^{AS} increased. The values are mean ± SD (n = 8; $^*P < 0.01$ compared with uninfected OCLs)

electrons from cytochrome *c* and four protons taken up from the mitochondrial matrix, without the formation of reactive oxygen species. The energy generated by the passage of electrons down the electron transport chain creates a proton gradient across the membrane that drives ATP synthase to make ATP from ADP.

To examine the functional consequences of the tyrosine phosphorylation of Cox by c-Src, we investigated whether or not c-Src kinase activity affected Cox activity. For this purpose, we used Src family tyrosine kinase-deficient mouse embryonic fibroblasts[37]. Cox activity in Src$^{-/-}$ Yes$^{-/-}$ Fyn$^{-/-}$ cells (SYF) was significantly decreased compared to Yes$^{-/-}$ Fyn$^{-/-}$ cells (Src++). Furthermore, c-Src re-introduction in SYF cells restored Cox activity to the higher level of Src++ cells.[22] These results lead us to speculate that c-Src can promote Cox activity in mouse embryonic fibroblasts. Further support for this conclusion was provided by an experiment showing that Cox activity in the enriched mitochondrial fraction was inhibited by the Src family inhibitor PP2. As in the SYF fibroblasts, Cox activity was reduced in c-Src$^{-/-}$ OCLs formed in vitro (Fig. 6A), providing further evidence that c-Src regulates Cox activity. To test whether modulating c-Src kinase activity in OCLs would affect Cox activity and bone resorption, we used adenovirus vectors containing kinase-dead Src (AxSrc^{K295M}), wild-type C-terminal Src family kinase (AxCsk^{WT}), or kinase-dead Csk (AxCsk^{KD}). Downregulation of c-Src kinase activity by Src^{K295M} or Csk^{WT} overexpression inhibited Cox activity in OCLs, while upregulation of Src kinase activity by AxCsk^{KD} infection induced higher Cox activity (Fig. 6B).[22] Thus, Cox activity is positively correlated with c-Src kinase activity.

Cox activity is correlated with osteoclastic bone resorption

To determine if reducing Cox activity affects the bone-resorbing activity of OCLs, we took advantage of the fact that nuclear-coded Cox subunit IV (CoxIV) is absolutely required for Cox activity.[38] So we constructed an adenovirus vector carrying CoxIV antisense (AxCoxIV^{AS}). The expression of CoxIV in OCLs infected with AxCoxIV^{AS} decreased in a dose-dependent manner. The decreasing levels of CoxIV correlated with decreased Cox activity in a biochemical assay; OCLs infected with AxCoxIV^{AS} showed 45% of basal Cox activity.[22] In addition, there was no significant change in c-Src kinase activity among OCLs infected with AxCoxIV^{AS}.

An actin ring was observed in 85% of the AxGFP-infected OCLs, whereas only 15% of OCLs infected with AxCoxIV^{AS} formed these rings (Fig. 6C). Consistent with the absence of the actin ring, bone-resorbing activity was progressively and severely inhibited by the infection with AxCoxIV^{AS} (Fig. 6D).[22] Interestingly, AxCoxIV^{AS} infection does not affect the survival of OCLs. These results strongly suggest that basal Cox activity is required for maintaining osteoclast morphology and for normal bone resorption. Consistent with these results, treating the cells with the classical Cox inhibitor KCN also decreased bone resorption. Diminished ATP levels by the failure of normal Cox could contribute to the observed loss of bone resorption Indeed, the complex I inhibitor rotenone and the complex

III inhibitor myxothiazol also prevented the bone-resorbing activity of OCLs in a dose-dependent manner, strongly suggesting that the rate of ATP generation by mitochondrial oxidative phosphorylation is critical for osteoclastic bone resorption. Furthermore, the inhibitory effect of $AxCoxIV^{AS}$ on bone resorption was not reversed by Csk^{KD} overexpression, even though c-Src kinase activity was increased in OCLs co-infected with $AxCoxIV^{AS}$ and $AxCsk^{KD}$ as much as it was in OCLs infected with $AxCsk^{KD}$ alone.[22] Taken together, these results indicate that Cox is a signaling effector downstream of c-Src that is necessary for bone resorption. Osteoclasts have a large number of mitochondria and express a high level of c-Src,[5,39,40] and a high level of ATP is required to support acid secretion by the osteoclast v-ATPase, as well as other functions that are required for bone resorption.[5] Thus, decreased Cox activity and the resulting reduction in ATP levels could explain, at least in part, the reduced bone-resorbing activity of $c\text{-}Src^{-/-}$ osteoclasts.

Concluding remarks

c-Src, which is highly conserved throughout evolution and widely expressed, associates with the cytoplasmic surface of cellular membranes. It is generally thought that c-Src's regulation of cell adhesion, movement, and proliferation involves its activity as a plasma membrane-associated molecular switch that links a variety of extracellular cues to specific intracellular signaling pathways. Our study demonstrates that both the formation of a Pyk2/Src complex, presumably in the podosomes, and the kinase activity of Src are required for bone resorption by osteoclasts. Elucidation of the role of Src in osteoclast function requires the identification of the signaling elements whose activities might to be modulated by the recruitment of active Src kinase to Pyk2. Src-catalyzed phosphorylation of Cbl on Tyr-731 is reported to create a binding site for the regulatory p85 subunit of phosphatidylinositol 3-kinase (PI3K),[41,42] and induce its activation and recruitment to the cell membrane. PI3K in turn is involved in a reorganization of the cytoskeleton that results in cell spreading and migration of several cell types including osteoclasts. Expressing the Cbl^{Y731F} mutant in osteoclasts markedly reduced their bone resorbing activity, suggesting that phosphorylation of Cbl-Y731 and the subsequent recruitment and activation of PI3K may be critical signaling events downstream of c-Src in osteoclasts. These results provide further insight into how the attachment of osteoclasts to the bone surface regulates bone resorption and how Src participates in this regulation.

c-Src is required for maintaining the basal Cox activity in mouse embryonic fibroblasts and osteoclasts. Furthermore, basal Cox activity is important for bone-resorbing activity of mature osteoclasts as well as c-Src. On the other hand, the most prominent defects of the SYF cells were reduction of cell proliferation and motility, and it should be noted that these are also ATP-dependent events. We therefore conclude that c-Src in mitochondria regulates Cox activity and that the c-Src/Cox signaling pathway is critical for the bone-resorbing activity of osteoclasts. The downregulation of Cox activity in the absence of c-Src may be involved in the osteopetrotic phenotype of $c\text{-}Src^{-/-}$ mice.

Interestingly, c-Src has also been reported to be present on late endosomes in fibroblasts,[43] synaptic vesicles in PC12 cells,[44] secretory vesicles in chromaffin cells,[45] vesicular structures in osteoclasts,[39,46] and the Golgi apparatus in CHO cells,[47] suggesting that c-Src is involved in multiple intracellular processes as well. In addition, Lyn, another Src family kinase, was found in rat brain mitochondria.[48] More recently, Itoh et al.[49] reported that Dok-4 recruits c-Src in mitochondria as an anchoring molecule and regulates NF-κB activation in endothelial cells. The subcellular fractionation also confirmed the localization of c-Src in all fractions, suggesting that c-Src associates with not only mitochondria but also various intracellular membranes. The identification and characterization of signaling cascades of c-Src on various intracellular membranes present an interesting new avenue for further elucidating Src's role in regulating cell function.

Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterized by invasive synovial hyperplasia. Proliferation of the synovial cells leads to pannus tissue that invades the bare area between cartilage and bone, finally resulting in progressive bone and joint destruction in the affected joints. The ultimate goal of the treatment of RA is to prevent bone and joint destruction and preserve the daily activity of the patients. Recent studies have shown that osteoclasts are involved in the pathogenesis of bone and joint destruction and can be a potent therapeutic target of this disease, and that therapies that inhibit osteoclast formation or function can at least ameliorate the progression of these bone changes. We have previously demonstrated that local injection of Csk virus, which negatively regulates Src family tyrosine kinases, into rat ankle joints with adjuvant arthritis not only ameliorated inflammation but also suppressed bone destruction. There will be no cure for RA until its etiology is elucidated, but suppression of osteoclast activity by regulating various intracellular signaling pathways including c-Src might lead to a novel therapeutic strategy for preventing the joint breakdown associated with RA.

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10

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連載

- ◎目で見るBone Biology
- ◎EBM講座
- ◎画像診断教室
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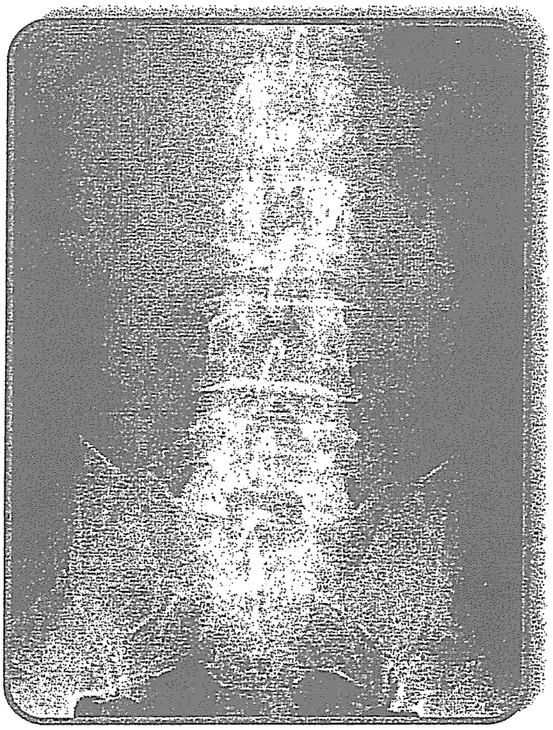

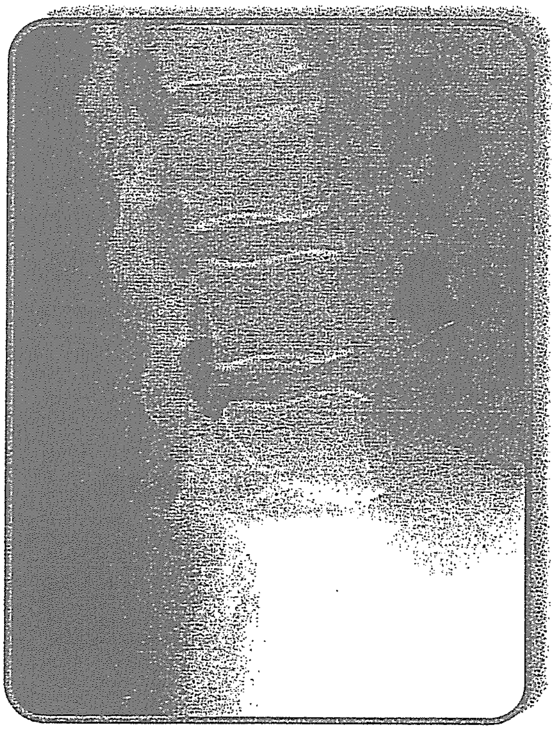

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先端医学社

アディポサイトカイン

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はじめに

脂質の過剰摂取・運動不足という現代の生活習慣は，過剰な脂肪蓄積を惹起し，メタボリックシンドローム発症の基盤となる．メタボリックシンドロームとは，腹部肥満すなわち内臓脂肪蓄積を必須に，高血圧，脂質代謝異常，耐糖能異常を伴う動脈硬化易発症病態を包括的にとらえた疾患概念である．一方，全身の白色脂肪組織が極端に減少する全身性脂肪萎縮症（脂肪萎縮性糖尿病）も，よく似た代謝異常症候群，すなわちインスリン抵抗性，糖尿病，脂肪肝，高脂血症を呈する．これは全身の糖・脂質代謝の恒常性を保つうえで脂肪組織が重要な役割を担うことを示唆している．

脂肪組織は重量として身体の10%以上を占め，人体における主要なエネルギー備蓄臓器と考えられてきたが，近年の分子生物学的アプローチにより，アディポサイトカインと総称されるさまざまな生理活性物質を産生・分泌する生体内最大の内分泌臓器であることが知られ，肥満，とくに内臓脂肪蓄積により惹起されるアディポサイトカインの分泌異常が，肥満症，脂肪萎縮症，両疾病における病態やメタボリックシンドロームと密接にかかわることが明らかとなった．

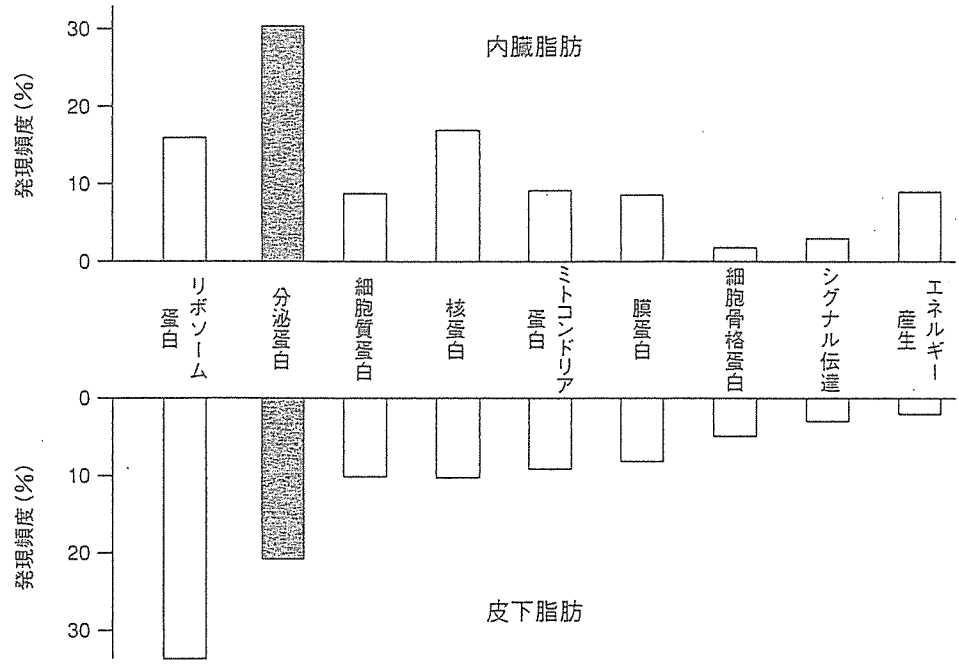
本稿では，生活習慣病あるいはメタボリックシンドロームを考えるうえで重要な4つのアディポサイトカインについて述べる（図❶，❷）．

関連語
- メタボリックシンドローム/生活習慣病
- 脂肪組織/生理活性物質
- アディポネクチン/レプチン
- PAI-1/TNF-α

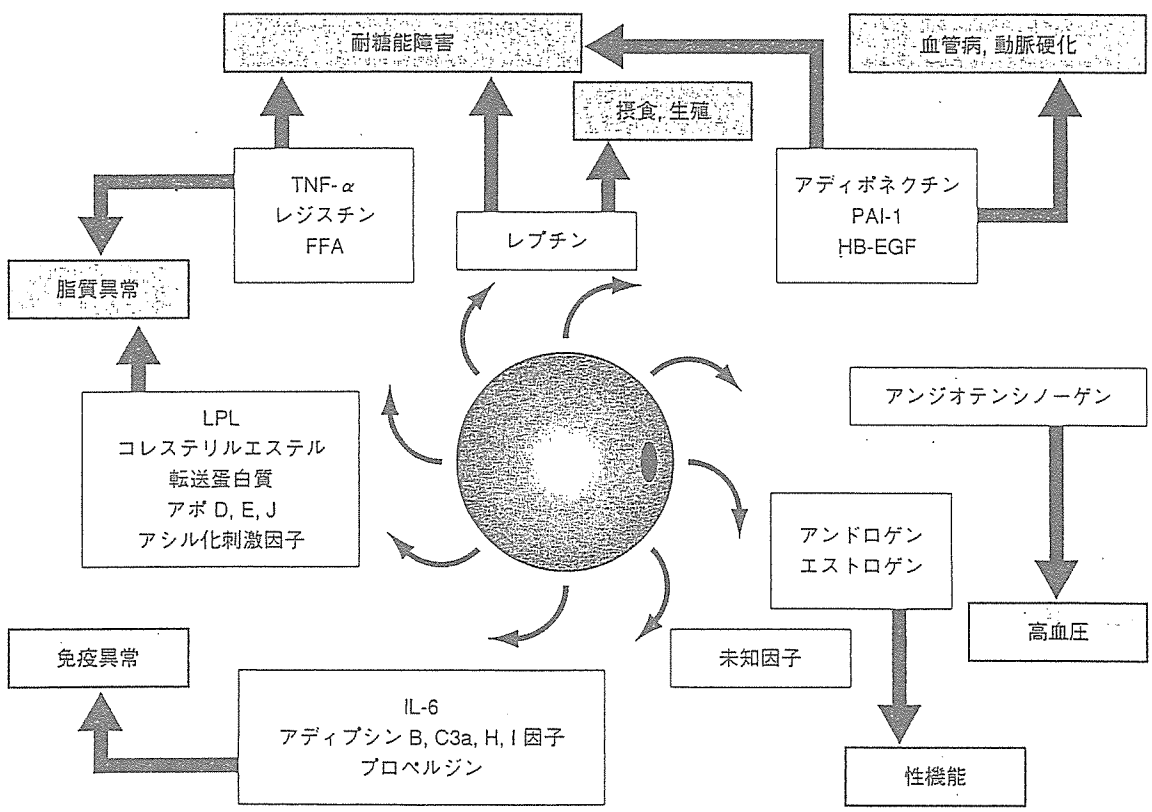
アディポサイトカイン

1）Plasminogen activator inhibitor-1（PAI-1）

生体内における血液の凝固と線溶は種々の因子によりバランスが保たれるが，plasminogen activator inhibitor-1（PAI-1）はプラスミノゲンアクチベータを抑制し，プラスミン生成を妨げ，フィブリンからのフィブリノーゲン分解産物生成を低下させる．つまり，PAI-1の増加は線溶活性を低下させ，血栓形成傾向に傾く．肥満者で静脈血栓症や心筋梗塞など，血栓性疾患の頻度が高いことは周知の事実であり，実際，脂肪蓄積，とくに内臓脂肪蓄積に伴い，脂肪組織PAI-1遺伝子発現量は上昇し，この上昇に連関して血中PAI-1濃度も上昇する[1]．血中PAI-1濃度は，肥満者，2型糖尿病患者で上昇し，血清トリグリセリド値とも相関する一方で，静脈血栓症や心筋梗塞患者でも上昇を認める．また，以前は血中PAI-1のおもな産生場所は肝臓であるとされていたが，脂肪蓄積に伴うPAI-1発現量は肝臓にて変化を認めず，内臓脂肪にて肝臓のレベルを凌駕する上昇を認めた[2][3]．これにより，肥満形成に伴い，蓄積脂肪から直接分泌される血中PAI-1の上昇が，肥満と血栓性疾患とを直接結びつける因子



図❶ ヒト脂肪組織発現遺伝子解析(Body map project)



図❷ 脂肪組織由来生理活性物質(アディポサイトカイン)とその作用
FFA : free fatty acid, HB-EGF : heparin binding-EGF like growth factor, LPL : lipoprotein lipase, TNF-α : tumor necrosis factor-α , IL-6 : Interleukin-6, PAI-1 : plasminogen activator inhibitor-1.

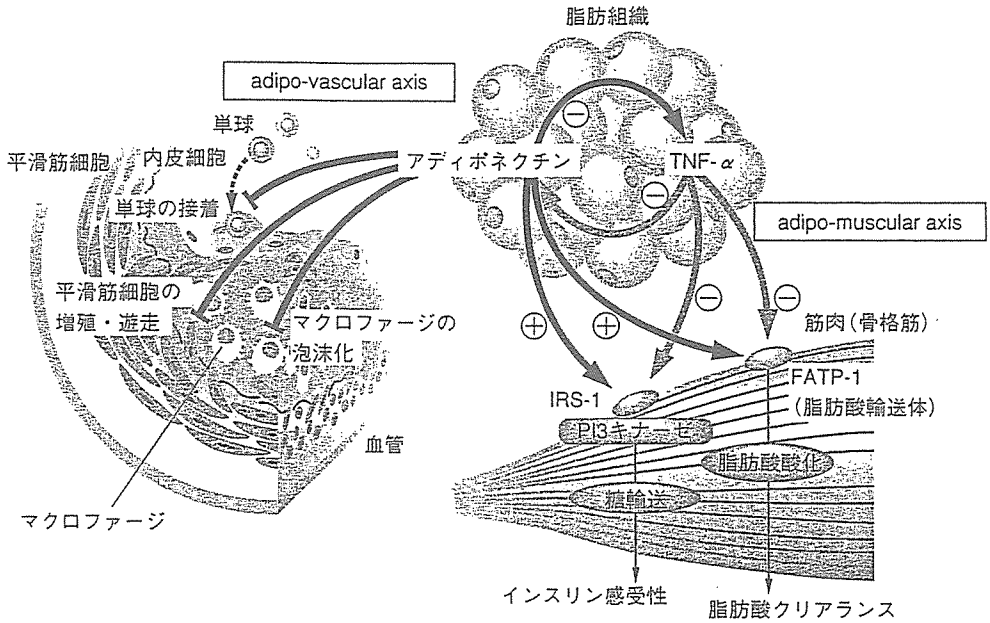
であることが示された．ヒトにおいて血中PAI-1濃度と脂肪分布の関連を調べたところ，血中PAI-1値は内臓脂肪面積と正相関を示すが，皮下脂肪面積とは相関しないことが明らかになった[4]．以上の知見から，内臓脂肪から直接分泌されるPAI-1が血中レベルに影響を与え，血栓性疾患の発症に関与するという，内臓脂肪蓄積と血管病変をつなぐ新たな道(adipo-vascular axis)の存在が明らかになった．また，最近，高脂肪食負荷による肥満・インスリン抵抗性の出現に対してPAI-1欠損マウスが抵抗性であること[5]，PAI-1がインスリンシグナルを抑制することが報告され[6]，肥満時の血中PAI-1レベルの上昇は肥満・インスリン抵抗性の病態発症そのものにかかわっている可能性もあり，興味深い．

2) Tumor necrosis factor (TNF)-α

Spiegelmanら[7]は遺伝性肥満動物の脂肪組織におけるサイトカイン遺伝子発現において，腫瘍壊死因子(tumor necrosis factor：TNF)-αが著明に増加していることを見出した．これら動物にTNF-αの中和抗体を投与したところ，インスリン抵抗性・糖尿病の改善がみられた．脂肪細胞や肝細胞においてTNF-αは，スフィンゴミエリナーゼの活性化を介して，インスリン受容体の基質であるインスリン受容体基質(insulin receptor substrate：IRS)-1のセリン残基をリン酸化し，チロシン残基のリン酸化を抑制することで，インスリン作用を減弱させる[8]．蓄積した脂肪組織より分泌されたTNF-αが筋肉，脂肪組織，肝臓での糖利用亢進を抑制し，インスリン抵抗性を介して，糖・脂質代謝異常をもたらすと考えられている．また，TNF-αは後述するアディポネクチンの遺伝子転写を抑制することによりアディポネクチンの産生・分泌を減少させる作用を有する(アディポネクチンの項参照)[9][10]．さらに，TNF-α，インターロイキン(IL)-1を介して核内で増えたNF-κBが，直接 peroxisome proliferator-activated receptor(PPAR)γに作用し，その転写活性を不活性化することが，脂肪細胞分化抑制につながることが明らかとなった[11]．TNF-α，IL-1のような肥大脂肪細胞から産生・分泌される因子が，未分化細胞の脂肪細胞への分化を抑制するという点は，アディポサイトカインが脂肪組織そのものに作用して代謝の恒常性・病態にかかわることを示唆しており，興味深い．

3) レプチン

Friedmanら[12]は肥満の遺伝的基盤を明らかにするため，ポジショナル・クローニングを用いて遺伝性肥満マウスの原因遺伝子の単離を試み，脂肪組織特異的な分泌蛋白質で，脂肪蓄積とともに脂肪細胞より分泌されるレプチンを同定した．その作用はおもに視床下部食欲中枢に作用し，食欲の抑制作用，エネルギー消費増強作用を介して，体重を減少させる．全身性脂肪萎縮症モデルマウスやレプチン欠損マウス(ob/ob)において認められるレプチンの欠乏は，糖・脂質代謝異常を招き，重篤なインスリン抵抗性，糖尿病，脂肪肝，高脂血症を発症するが，レプチン補充にて，改善もしくは正常化する[13]．レプチンの全身性脂肪萎縮症への有効性はすでにヒトにおいても証明され，糖尿病，高脂血症，脂肪肝の劇的な改善に至っている[14]．その作用機序として，レプチン欠乏が引き起こす慢性的な高インスリン血症が，肝臓の脂肪酸・中性脂肪合成を上昇させる転写因子SREBP-1cを上昇させ，脂肪肝，高脂血症を引き起こすこと，高インスリン血症が肝臓の糖代謝に重要なアダプター蛋白IRS-2の絶対量を減少させることでインスリン抵抗性を引き起こし，定常的に糖の産生・放出が上昇し高血糖へとつながることが考えられている[15]．また，レプチンには交感神経系を介した昇圧作用があり，肥満時には脂肪組織からのレプチン産生が上昇することから，肥満者の高血圧には高レプチン血症の関与が考えられる．さらに，レプチンは視床下部—交感神経系を介して，かつ骨格筋に直接作用して，骨格筋での脂肪酸酸化を亢進させることが報告され[16]，この作用がAMP-activated protein kinase (AMPK)を介して惹起されることを見出し，Leptin-AMPK axisが骨格筋におけるエネルギー消費の重要な制御機構であることを示した．レプチンは，骨代謝に及ぼす作用も報告されている．中枢性に骨形成を抑制すること[17]，逆に，骨芽細胞に直接作用して，石灰化や増殖分化を促進すること[18][19]や破骨細胞前駆細胞に作用してその分化を抑制することなどが報告されている[20]．



図❸ アディポネクチンの作用(adipo-vascular, adipo-muscular axis)

4) アディポネクチン

a. アディポネクチンの発見と血中濃度変化

アディポネクチンは，Body map project[21]におけるヒト脂肪組織遺伝子ライブラリーに最も頻回に出現し，脂肪組織特異的に発現した遺伝子adipose most abundant gene transcript 1(apM1)の産物である[22]．血中アディポネクチン濃度は5～30 $\mu g/ml$と高濃度に存在し，肥満者や男性において低下し，減量によって増加する[23][24]．さらに，心筋梗塞や狭心症といった動脈硬化性疾患，糖尿病で低下する[25][26] (図❸)．

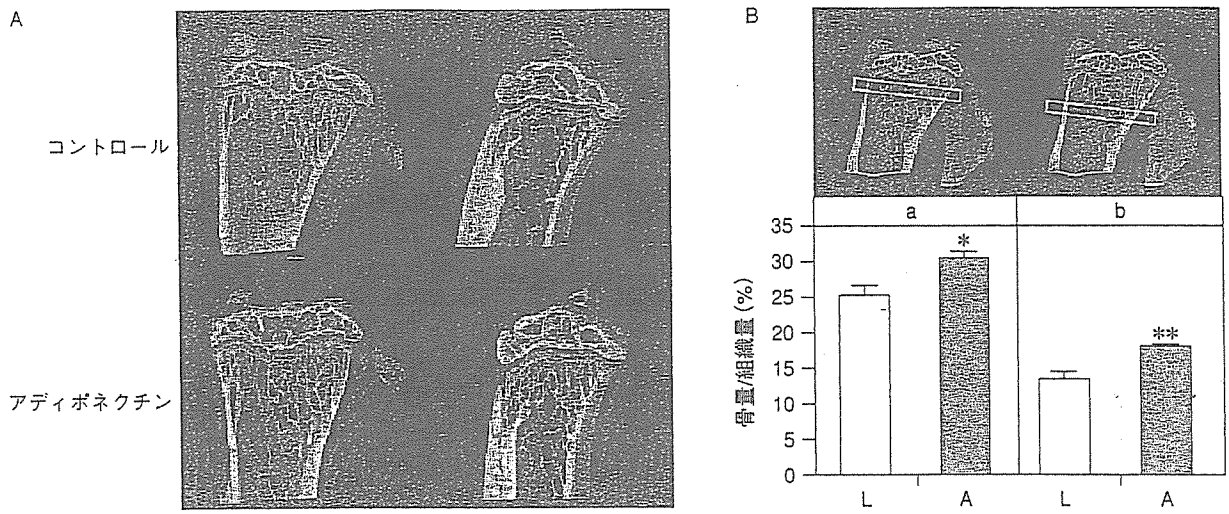
b. アディポネクチンの抗動脈硬化作用 (adipo-vascular axis)

アディポネクチンは，血管内皮細胞においてTNF-α依存性に上昇する接着分子VCAM-1，ICAM-1，E-セレクチンの発現を抑制し，血管内皮細胞と単球との接着を阻害する[25]．また，マクロファージの泡沫化や血管平滑筋細胞の増殖を抑制する[27][28]．これらはアディポネクチンが抗動脈硬化ホルモンであることを示す．

最近報告されたcase-control studyでは，入院時急性心筋梗塞や急性冠症候群と診断された症例は，安定型狭心症と診断された症例にくらべ，血中アディポネクチン濃度が有意に低値であり[29]，マクロファージにアディポネクチンを添加したところ，プラーク攻撃因子であるmatrix metalloproteinase-9(MMP-9)の発現は変化しないが，これに拮抗する防御因子であるtissue inhibitor of metalloproteinase-1(TIMP-1)の著明な増加がみられた．これは，アディポネクチンが動脈硬化の発症進展を抑制するだけでなく，プラーク破綻に対しても抑制的に作用していることを示唆する[30]．

c. アディポネクチンの抗糖尿病作用(インスリン感受性増強作用)

血中アディポネクチン濃度は全身のインスリン感受性と強く相関する[31]．アディポネクチンは，筋肉細胞にはたらいて，IRS-1シグナリングを介したPI3-kinaseの活性および糖輸送を上昇させ，インスリン感受性を増強させる．さらに，アディポネクチンは脂肪酸輸送蛋白1型(fatty acid transport protein 1：FATP-1)の遺伝子発現増強を介して脂肪酸の酸化およびクリアランスを高め，インスリン感受性を上昇させる[32]．そして，脂肪蓄積で脂肪組織より過分泌されるTNF-αは同経路に作用してアディポネクチンと逆の作用を示す．一方，アディポネクチンとTNF-αは，互いの作用を抑制しあうだけでなく，その産生場所である脂肪組織において転写レベルでの調節によりお互いの産生を抑制しあうことがわかった．つまり，



図㊾ アデノウイルスを用いたアディポネクチン強発現マウスの3D-μCT解析
A：3D-μCT画像．B：海綿骨量(脛骨)，成長板より50～250μm(a)，500～700μm(b)の領域．L:コントロール，A：アディポネクチン．

アディポネクチンは，インスリン抵抗性惹起因子であるTNF-αの産生と機能を抑制する．実際，糖尿病モデルマウスにアディポネクチンを補充すると血糖値が下がることが報告され[33][34]，アディポネクチンのインスリン抵抗性改善作用は，骨格筋，肝臓で脂肪酸酸化に重要なはたらきをしているAMP kinaseを介したものであることが明らかにされた[35]．

d．遺伝的低アディポネクチン血症からの知見

アディポネクチン欠損マウスは，高脂肪・高ショ糖食2週間負荷にて，強いインスリン抵抗性・糖尿病を呈し，アデノウイルスによる血中アディポネクチン補充により野生型レベルまで改善した[32]．血管病変については[36]，大腿動脈擦過後の血管内膜平滑筋の増殖すなわち内膜肥厚の進行を認めるも，補充にて野生型レベルまで改善した．アディポネクチン欠損マウスは，外観上正常と変化ないが，環境負荷や傷害に対して強く反応が起こるという，生体侵襲に対する防御因子としての本分子の一面がうかがえる．

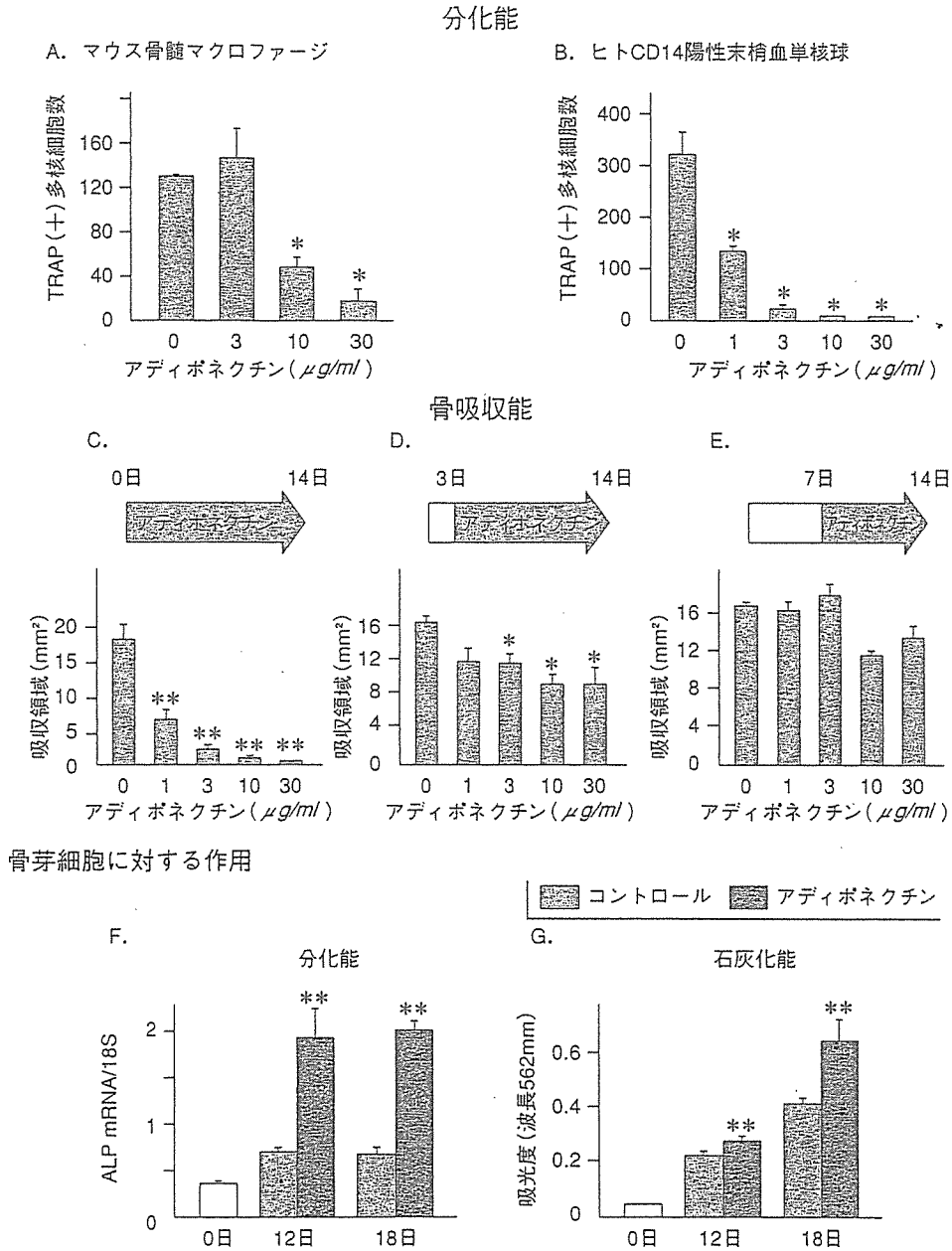
現在までヒトにおいて本分子を完全に欠損する遺伝子異常は報告されていないが，興味深いのは，血中アディポネクチン濃度の低下を伴うI164T変異で，耐糖能異常，高脂血症，高血圧や動脈硬化疾患を高率に合併し，まさにメタボリックシンドロームの表現型を示す[37]ことである．

e．炎症，高血圧，肝線維症-生活習慣による多彩な病態への関与

近年，動脈硬化疾患や2型糖尿病発症の基盤として，炎症が注目されている．アディポネクチンはTNF-α分泌を介して炎症と関連するが，血中high sensitive CRP(hsCRP)濃度はアディポネクチン濃度と弱い逆相関関係にある．アディポネクチン欠損マウスでは脂肪組織CRP発現量が増加しており[38]，この組織が多くの補体分子を発現していることから，過栄養に対するバッファー機能のみでなく，炎症に対する防御機能を有していると考えられる．

高血圧はメタボリックシンドロームの重要な病態である．一般集団で血中アディポネクチンとの相関関係をみると，HDLコレステロール，トリグリセリド濃度やインスリン抵抗性指標にくらべ，血圧との関連は低い．しかし，本態性高血圧群をみると血中アディポネクチン濃度は血圧と逆相関する．アディポネクチン欠損マウスに高脂肪高食塩負荷をおこなうと，血管内皮機能が障害され血圧は上昇する[39]．アディポネクチンは，血管内皮機能を介し高血圧へも関与していると考えられる．

動脈硬化は一種の過剰な創傷治癒反応である．アディポネクチン欠損マウスに四塩化炭素を投与した肝臓障害実験では[40]，野生型にくらべ著明な肝線維化が起こり，線維合成にあずかる肝星細胞(α-smooth muscle actin陽



図⑯ 培養細胞に対するアディポネクチンの作用
アディポネクチンによる破骨細胞の分化(A, B)・活性化(C, D, E)抑制作用および骨芽細胞の分化(F)・活性化(G)促進作用を示す.
A-E：誘導破骨細胞初代培養(マウスおよびヒト細胞), F-G：誘導骨芽細胞培養(MC3T3-E1細胞), TRAP：酒石酸抵抗性酸ホスファターゼ, ALP：アルカリホスファターゼ.
Studentのt検定によるP値：＊p＜0.05, ＊＊p＜0.01.

性細胞)が活性化され, TGF-β1, connective tissue growth factorなどの線維化関連遺伝子の発現が上昇していた.

f. アディポネクチンの骨代謝に及ぼす影響

アディポネクチンレセプターが同定され, その発現がユビキタスであったことから, レプチンのみならずアディポネクチンも骨代謝に何らかの影響を及ぼすことが容易に想像された. 最近, *in vivo*の検討にて, アデノウイルスを用いたアディポネクチン強発現マウスの海綿骨量が著明に増加し, 破骨細胞数の減少とNTxの減少を伴うことが明らかとなり[41], *in vitro*では, アディポネクチンが

破骨細胞の分化，吸収活性を抑制すること，骨芽細胞の分化，石灰化を促進することが明らかにされた[41]（図❹，❺）．また，骨芽細胞の増殖・分化促進作用は，MAPKシグナルを介したものであることがわかった[42]．

おわりに

近年における臨床的，基礎的両側面からの研究アプローチにより，メタボリックシンドロームの発症基盤として，脂肪組織，とくに内臓脂肪蓄積の意義は確立した．今後，内臓脂肪蓄積とそれに引き続くアディポサイトカイン産生・分泌異常を病態マーカーとして早期にとらえ，治療標的として臨床応用することが，個々のリスクファクターに対する治療を超えたメタボリックシンドロームの包括的な予防および治療につながると考えられる．

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第2章　骨代謝の基礎

肥満とカルシウム骨代謝*

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はじめに

臨床的に，肥満すなわち体重による力学的負荷が骨量と相関があることは広く知られているが[1]，肥満による脂肪組織増大やエネルギー代謝変化による生理活性物質の分泌変化が骨代謝にどのような影響を及ぼしているかはいまだ一定の見解を得ていない．しかし，脂肪組織が内分泌臓器であるという概念が提唱されて以降，脂肪組織，肥満と骨代謝に関する研究が急速に進められている．

肥満と骨代謝の関与

肥満と骨代謝に関与すると考えられる因子は物理学的因子と液性因子の2つに分けられる．物理学的因子とは体重など骨への機械的刺激による力学的負荷であり，液性因子とは脂肪組織由来生理活性物質(アディポサイトカイン)，脂肪組織で代謝されるエストロゲン，膵β細胞由来生理活性物質であるインスリンなどである．そして，体重や筋肉量の増加による骨への機械的刺激により皮質骨の肥厚や骨サイズの増大が生じ，ホルモンやサイトカインなどの液性因子により海綿骨量の増加が生じると考えられている．

肥満は加齢に伴う骨喪失率が低く，体重増加に伴い骨量が多くなることが一般的に知られている．また，成長期や思春期の肥満は骨密度の上昇，骨サイズの増大をひき起こすことが報告され，逆に，骨粗鬆症のスクリーニングで，やせは重要な危険因子になることが報告されている．

最近の臨床研究では，肥満による骨量増大には性差があり，男性ではlean body massの増加が，女性ではfat massおよびlean body massの増加が重要であると報告されている．また，最近の基礎研究では，脂肪組織から分泌されるレプチンやアディポネクチンなどのアディポサイトカインが骨に対して直接作用を有することが明らかとなった．

1．力学的負荷

骨は主にコラーゲンからなる有機基質とリン酸カルシウムを主体とする無機質からなる生体の支持組織であり，重力に抗した姿勢の保持や運動機能の維持の役割を果たしている．このため，骨はその各部位に負荷される外力に応じて外形および内部構造の形態を変化させている．同時に，骨は生体のカルシウムの99%以上を含有する巨大なカルシウム貯蔵庫として，体液中のカルシウム濃度の保持に関与している．すなわち，血中カルシウム濃度の低下に伴い副甲状腺

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ホルモン(PTH)の分泌が増加し，骨に蓄積されたカルシウムが血中に溶出され，骨吸収が起こる．さらに，PTHにより産生される1,25-$(OH)_2D_3$の働きでカルシウム結合蛋白質の合成が増加し，腸管からのカルシウム吸収が促進され，再び骨にカルシウムが補充され，骨形成が起こる．つまり，骨は外力に応じて形態を変化させ強度を維持しながら，血中カルシウム濃度を保持するための貯蔵庫として働き，必要に応じてカルシウムを体液中に供給するという機能をもつ．このため，骨は単なる剛体ではなく，力学的負荷の少ない部位では骨吸収が優位となり，強度を保つ必要のある部位では骨形成が優位となるように，常にリモデリングを繰り返している．

これまでに，体重と骨量の間に正の相関関係があることが知られ，肥満により体重が力学的負荷となることで骨量は増加し，逆に，長期臥床や宇宙船内での微小重力環境により骨量は減少する(不動性骨粗鬆症)とされている．また，閉経後女性において，肥満は骨量低下を予防する効果があり[2]，脂肪組織量は骨密度に正の相関関係を認めている[3]．

力学的負荷がどのように骨量増加をひき起こすかは不明な点が多く，骨芽細胞，破骨細胞または骨細胞の力学的負荷の感知方法やシグナル伝達は解明されていない．骨芽細胞，破骨細胞，骨細胞による感知方法として，骨微小管内液流変化によるfluid shear stressや細胞の伸展刺激，静水圧などがあげられ，力学的負荷による細胞内シグナル伝達として，細胞膜上のストレス感受性陽イオンチャンネルを介したチロシンキナーゼ活性化やインテグリンの関与の報告がある．

2．サイトカイン

加齢に伴い骨粗鬆症が進行し骨量が減少する一方で，骨髄脂肪細胞は逆に増加する[4]．骨芽細胞と脂肪細胞はともに骨髄に存在する共通の未分化間葉系幹細胞から分化することが知られ[5]，この2つの細胞への分化のスイッチングにはかなりの可塑性が存在するとされるが，分子メカニズムに関してはよくわかっていない．

脂肪細胞分化に必須の転写因子としてPPARγが知られ，PPARγ欠損ES細胞は脂肪細胞へは分化しないが，骨芽細胞へは自発的に分化することが報告されており，PPARγノックアウトマウスでは，ホモは胎生致死であるがヘテロは正常に成長し[6]，野生型に比し骨量増加を認め，加齢に伴いその効果がより強くなると報告されている[7](図1)．

骨代謝および脂肪代謝を制御するホルモンはPPARγシグナルと関連しており，PPARγ減少により骨髄の幹細胞から骨芽細胞への分化が促進され骨量増加をきたすことから，脂肪組織，脂肪細胞の変化がサイトカインなどを介して骨代謝に影響を及ぼすことは想像に難くない．脂肪細胞は多くのアディポサイトカインを分泌し，それらは肥満すなわち脂肪組織，脂肪細胞の肥大に伴い分泌量が変化することで骨代謝に影響を及ぼすことが容易に類推される．

(1)エストロゲン

エストロゲンは女性ホルモンとして知られ，閉経前は主に卵巣で産生され，閉経後は脂肪組織をはじめとする末梢組織で副腎由来アンドロゲンから変換される．また，エストロゲンは骨髄細胞に直接作用して破骨細胞への分化を抑制し，骨芽細胞の増殖，骨基質蛋白の産生を促進する．それゆえ，閉経後に卵巣機能が低下し，エストロゲンが欠乏すると，骨代謝回転が亢進し，骨吸収促進による骨量減少が生じるが(閉経後骨粗鬆症)，脂肪組織が多ければ多いほど，副腎由来アンドロゲンから変換されるエストロゲンが増えるので骨量維持には有利と考えられている．

実際，Tremolliresらは，閉経後早期の腰椎の骨量減少率は，過体重群(BMI＞25)では正常体重群(BMI＜25)より少なく，骨量減少率とBMIに有意な相関があったと報告している[8]．

(2)インスリン(図2)

肥満はインスリン抵抗性を惹起する最大の因子である．インスリン抵抗性が生じると，インスリンによる肝臓の糖放出抑制作用，末梢組織(骨格筋，脂肪組織)への糖取り込み作用が障害され，インスリンの血糖降下作用が発揮されないため，インスリン分泌予備能が保たれていれば，代償性にインスリン分泌が亢進して高インスリン血症となる．このインスリンおよびインスリン様成長因子-I (insulin-like growth factor-I；