原著論文

一般高齢者がもつアルツハイマー型認知症についての知識量と関連要因の検討

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抄録

本研究では，一般高齢者がもつアルツハイマー型認知症(Dementia of Alzheimer's type；DAT)についての知識量および認知症のイメージや自分自身が認知症になる不安感と，それらに関連する要因について検討することを目的に，京都府下の生涯学習センターの受講生188人を対象として調査を実施した．その結果，5割以上の人がDATの周辺症状や治療薬について誤った認識をもっており，高年齢になるほど知識が低くなることが示された．また，認知症に対して「病気ではない」というイメージをもつ人が7割を超えていた．自分自身が認知症になる不安感は8割の人が，わずかあるいはそれ以上あるとしていた．今後，正しい知識や情報を提供し，認知症の正しい理解をうながし不安感を軽減させていく必要がある．認知症専門外来も啓発活動の重要な担い手となるべきであるが，認知症専門外来そのものの認知率は約3割であり，周知に向けた努力が必要であろうと考える．

Key Words：アルツハイマー型認知症，知識，イメージ，不安感，認知症専門外来

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緒　言

現在，わが国にはおよそ160万人以上の認知症患者がいるといわれているが，さらに増加の一途をたどることは確実であり，多くの人々にとって自分自身あるいは家族が認知症になるということが，身近な問題になってきている．今日，アルツハイマー型認知症(Dementia of Alzheimer's type；DAT)については，医学・医療的見地から発症のメカニズムの解明や治療薬および早期診断技術の開発が，また福祉的見地から介護方法や介護サービス等の整備が，さらには社会的見地から認知症高齢者に対する見方等についての研究など，多方面から研究や対策が急速に進んでいる．しかし，それらの知見が一般の人々に周知されているかといえばそうとも限らない現状がある．さらに，TVや書籍等のメディアにより散発的に伝えられる情報は必ずしも正しい理解につながっているとはいえず，かえって不安感を助長している可能性もある．

一方，現在認知症を本人に告知するか否かが大きな課題となりつつある．認知症の告知に対する一般の人々や介護者の態度についての研究もいくつかなされているが，その是非や理由についてたずねたものがほとんどである[1-3]．認知症の告知について検討する際，告知を受ける側である患者やその家族になる可能性をもっている一般高齢者が，認知症はどのような病気であると認識し，認知症になった際にどのような力が残され，どういった状態になると考えているのかという点について把握しておくことは，より差し迫った事態になったとき，どのような反応を生じるかを予測するうえで重要であると考える．

海外においては介護専門職や家族介護者および一般大衆の認知症に対する知識を評価する重要性

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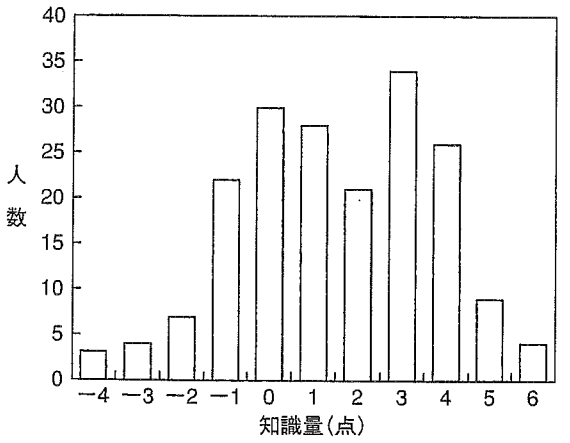
表 1　調査対象者の基本属性

		対象者数(%)
性　別	男性	126(67)
	女性	62(33)
年齢別	69 歳以下	120(63.8)
	70 歳以上	68(36.2)
結　婚	未婚	2(1.1)
	既婚	160(85.1)
	離別・死別	26(13.8)
家族形態	独居	19(10.1)
	夫婦のみ	98(52.1)
	2・3 世代同居	69(36.7)
	その他	2(1.1)
認知症介護経験	あり	31(16.5)
	なし	157(83.5)

調査対象者 188 人の基本属性について示す.



アルツハイマー型認知症についての知識量を点数で表しその人数を示す(n=188)

図 1　アルツハイマー型認知症についての知識量

でも選択できることとした.

統計学的処理には SPSS10.0J を用い，2 群間の検定には t 検定，χ^2検定を行った.

Ⅱ. 結　果

調査対象者の基本属性を表 1 に示す.

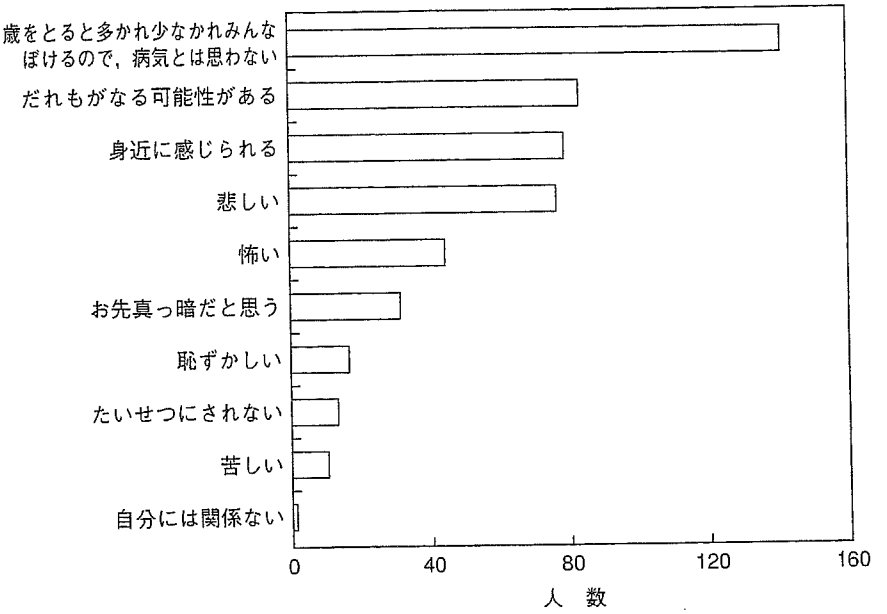
DAT の知識量では 7 問が正解であるため，理論上の最高点は 7 点となり，最低点は−11 点となる. 結果は図 1 に示したように，最高 6 点，最低−4 点であり，平均 1.52 点(SD=2.20)であった. 設問に丸印をつけた数は 1 人当たり平均 7.38 個(SD=2.50，範囲 2〜13)であった.

各設問の積極的回答の正誤については，表 2 に示す. DAT の一般的知識について，認知症の原因疾患，発症年代，原因の解明についての正解率はそれぞれ 77.7%，70.7%，52.7%で，原因の解明がどの程度進んでいるのかについての認識にはばらつきがみられた. 一方，老化との関連について，DAT が脳の老化によりだれもがなると誤答していたのは 14.4%であった.

DAT の症状のなかで，中核症状についての知識では，失見当識，判断力の低下についての正解率はそれぞれ 67.0%，79.8%であった. 一方，DAT でみられる障害が記憶障害のみであると誤答しているのは 4.3%とわずかであり，早期から人格が崩壊する，同じことを何度も聞くようになると重症であると誤答していたのはそれぞれ 20.2%，38.8%であった. 周辺症状の知識では，もの盗られ妄想がでてくることもよくあると正解したのは 48.4%であり，徘徊行動がでる場合が多いと誤答していたのは 62.2%と 6 割を超えていた. 早期の段階の行動能力についての知識では，身の回りのことがほとんどできなくなる，金銭管理は不可能，独居は不可能と誤答していた人は，それぞれ 14.4%，23.4%，38.3%であった.

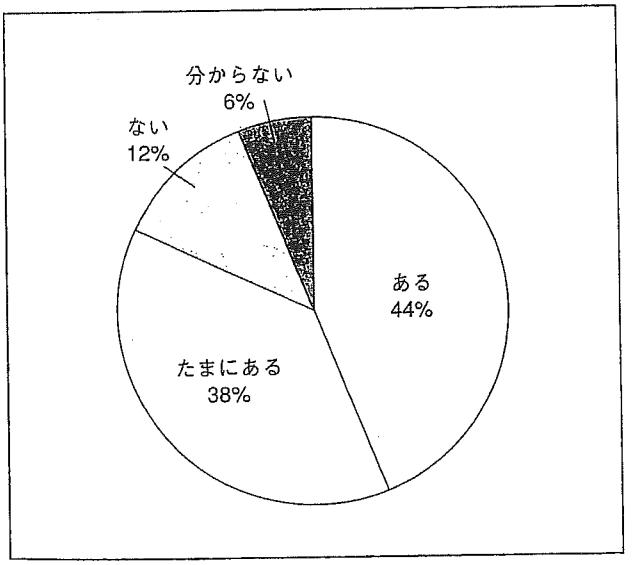
DAT の治療についての知識では，治療薬の有無についての正解率は 48.4%であったが，17.6%の人が現在治療法はまったくないと誤答していた. 早期治療は効果がない，周囲の対応によっても問題行動は軽減しないと誤答しているのはそれぞれ 20.2%，17.0%であった.

性別で DAT についての知識量の差をみると，男性の平均 1.4 点(SD=2.30)，女性の平均 1.74 点(SD=1.97)であり，有意な差はみられなかった. 設問ごとにみてみると，認知症の原因疾患についての設問では，女性の正解率 90.3%，男性 71.5%と女性の正解率が有意に高かった($p<0.01$). 一方，早期治療は効果がないと認識している人は男性 15.9%，女性 29%と女性に多かった($p<0.05$).



認知症に対するイメージの各項目に丸印をつけた人数を示す．回答は複数回答可（n＝188）

図 2　認知症に対するイメージ



自分自身が認知症になることへの不安感について“ある”“たまにある”“ない”“分からない”と答えた割合を示す．

図 3　自分自身が認知症になることへの不安感

は33.0%であった．性別，年齢で認知に有意な差はみられなかった．

Ⅲ．考　　察

今回の研究では，DATが自分自身あるいは家族にとって差し迫った問題となるであろう一般高齢者が，DATについてどんな認識やイメージをもっているかを把握することを目的とした．認知症に対するイメージのなかで，「自分には関係ない」と答えた人が188人中1人のみであったことからも，ほとんどの人が自分自身のこととしてとらえていることがうかがえる．

調査の結果，周辺症状についての知識が低いことが示された．周辺症状の1つとしてもの盗られ妄想がよくあることへの認識が低かったことは，実際にもの盗られ妄想の症状に直面した介護者が，それを認知症の症状とは結びつけることができず，介護者に対する悪意と誤解したり，さらには攻撃とまで受け取ってしまうという現象例の1つの原因とも考えられる．また，徘徊行動が頻発すると認識している人が多いことについては，DATの症状のなかで徘徊行動が一般の人々の目にも触れやすい症状であることや，マスメディア等の情報において徘徊行動を強調するようなものも見受けられるため，そのような認識をもつ人が多かった可能性が考えられる．さらに，中核症状の知識でも，早期の段階から人格が崩壊すると誤って答えた人は2割にとどまったが，「同じことを何度も聞くのは重症」と誤って考えている人

あった．本間の調査では若年層を多く含むことから，あまり差し迫った問題とは考えておらず，自分自身が認知症になる不安が4割程度にとどまったものと思われる．さらに，今回の調査では本間のものより知識の正答率が高いことから，知識が増えたことにより，イメージや不安感に影響を及ぼした可能性も考えられる．この関連性の分析は今後の研究に期したい．

今後，正しい知識や，治療および対応方法についての情報を提供し，認知症の正しい理解をうながし不安感を軽減させていく必要があると思われる．これらの啓発活動を多方面から行う必要があるが，認知症専門外来も重要な担い手の1つとなるべきであろう．しかし認知症専門外来そのものの認知も浸透しているとは言い難く，今後周知に向けた努力が必要であろうと考える．

次に介護経験の有無による影響については，Werner が行った調査によると，アルツハイマー病についての知識の低さ，とくに病気の原因や症状についての知識が低かったことが指摘されている[6]が，介護者のみを対象にしており，介護していない人との比較におけるものではない．今回の調査では認知症の介護経験の有無で知識量に差はみられず，介護経験により必ずしも知識量は増えていないと考えられる．ただし，今回の調査では調査対象者である一般高齢者を，本人の申告による認知症介護経験の有無で分別したものであったので，現在あるいは過去に痴呆専門外来に通院している患者の主介護者といったような，より明確な形での認知症介護経験者への追調査が望まれる．いずれにしても，いかに介護者に必要な知識を提供していくかは重要な課題であり，そのことに患者や介護者と接する診療機関としての認知症専門外来等がどのようにかかわるかも問われるであろう．認知症についての知識水準が高い介護者ほど，うつの傾向は低いが不安感が高い傾向にあるという Graham らの報告[7]もあり，どの領域の知識がうつや不安につながるのかといった調査や，さらには Graham らも述べているように，うつも不安も最小限にとどめ得る教育のあり方の開発が望まれる．

今回の研究では，一般高齢者が DAT についてどんな認識をもっているかを把握することを目的としたが，測定方法にはさらなる検討が必要である．Dieckmann らや Gilleard らのスケールは，いずれも生物学的な内容の設問が多く，専門的で複雑なものであり，一般高齢者を対象に認知症についての知識を計るスケールとしてはやや不適切であると思われたので，本研究では本間の調査の設問を参考にし，DAT について重要と思われる内容の設問を加え調査を行った．しかし，今回の設問も必要十分とはいえず，改善の余地があると思われる．また設問によっては，有無のみをたずね明確に答えが出るものもあれば，その程度を含んだ設問であるため答えにあいまいさが残るものもあった．さらに，徘徊の定義や，早期の段階の独居をどの程度で不可能とみなすかによって，設問の正誤が変わる可能性もある．今後さらに検討を加えたい．

今回の調査では認知症の告知についての希望も合わせて調査したが，これらについては別稿で述べることとしたい．

認知症を患った人やその家族が病気を適切に受け止めたり，地域社会のなかで認知症の人のノーマライゼーションがはかられるためには，このような研究結果が参考にされ，より正確な知識が普及することを期待したい．

なお，本研究は日本興亜福祉財団ジェロントロジー研究助成を受け，「痴呆症の病名および予後の告知に関する研究」の一端として行った．調査にご協力いただいた，岡本民夫教授（同志社大学文学研究科社会福祉学専攻）ならびにアンケートの回答者の皆さまに深く感謝申し上げる．

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はじめに

センター方式が認知症の人のためのケアマネジメントツールとして本格的に使用されることになったが，認知症という病気を診療する立場から認知症ケアの新たな進歩を喜ぶと同時にセンター方式が医療との連携を今まで以上に深めるツールになることを期待している。

センター方式の評価と今後の課題 ―医療の立場から―

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KEY WORDS
◆認知症の障害
◆認知症ケアの方法論
◆介護と医療の連携
◆早期からの導入
◆本人・家族の参加

私は同僚らと介護保険開始の１年前，老年内科に物忘れ外来を開設し，認知症の早期発見，早期介入に取り組んできた[1]。また，院内の地域ネットワーク医療部という地域の医療・福祉施設との連携を深め患者の立場を支援するソーシャルワーク部門の立ち上げに参加した。そして，認知症の知識を一般市民に周知するため市民講座や保健所講座を行ったり，介護実務者講習の講師を務めたりしてきた。その関係から，平成16年度，京都市がセンター方式のモデル事業に全国16地域の一つとして参加する際，地域検討委員としてこの事業に参加させていただいた。地域でのセンター方式学習会や事例検討，地域および中央での報告会などを通じてその成果を見ることができた。これらの経緯を踏まえて，医療の立場からセンター方式の位置づけや可能性，課題について論じたい。

間だけでその人の状態を把握することは困難な場合が多い。C-1-1「心身の情報」，D-1「できること・できないこと」，D-2「わかること・わからないこと」，D-3「生活リズム・パターン」，D-4「24時間生活変化」などの情報があれば，主治医も全体を把握した上で検査や処方を適切に行うことが可能であろう。例えば「興奮や易怒性が目立つのでどうにかなりませんか」と介護者が強く訴えてきた場合でもD-4シートで穏やかな時間も多いことを確認して投薬による対処を控えたり，D-3シートで睡眠や排泄など体調面での問題が浮き彫りになれば，その点の改善に集中することができるであろう。これらはほんの一例でありセンター方式の情報が医療者の判断に役立つ場面は多いであろう。従来，介護の現場から医療への情報発信が積極的に行われることは少なかったが，今後はセンター方式というツールを媒介に連携が強化されることを期待したい。

次に，医師・看護師・薬剤師の方から多くの役立つ情報をケアスタッフに提供することも可能であろう。A-3「私の療養シート」ではその人の持つ病気や飲み薬などについて詳しい情報があればケアの現場での援助は円滑になるだろう。D-1「できること・できないこと」，D-2「わかること・わからないこと」などのシートで，神経の障害の側面から「できること・できないこと」「わかること・わからないこと」がなぜそうなのか，それは神経の障害により不可避なものなのか，今後関わりによって変化する可能性があるのかなどを検討することができるであろう。D-3シートにおいても心臓や腎臓の機能との関係で水分や食事・排泄について相談が可能であろうし，睡眠の状態や睡眠薬などの副作用としてのふらつき・転倒の有無と睡眠薬・安定剤・抗うつ剤の使用の適否について相談が可能であろう。

ただ，これらの連携において，ケアスタッフとセンター方式シートについて議論するためにはセンター方式や新しいケアの進め方に関する医療関係者への研修も必要であろう。講習の参加により認知症ケア連携医などの資格を与える制度を創設し実質的な連携が行えるような工夫が望まれる。

2．早期からの導入と本人・家族の参加について

医療機関は多くの認知症患者と家族が最初に訪れるところであり，今後，早期発見の機運が高まればその傾向はさらに顕著になるであろう。そして，この時，患者自身も自分がどのように生きてきて，今後どのように生きたいのか，自分で判断する力を持っている場合は多い。また，家族も認知症患者への対応に疲れ切ってしまった状態ではなく，むしろ，肯定的に支えてあげたいという気持ちを持っていることも多い。モデル事業におけるセンター方式の大きな成果の一つとして「家族とスタッフの対話の増加」や「家族の認知症ケアへの理解向上」が見られたが，多くの家族や本人は介護保険サービスを利用するまでにいろいろと悩んでいる。特に，施設での介護に委ねるときは大きな決断を迫られている。ローリー・ホワイトとベス・スペンサーの「高齢者のお引っ越しガイド」[8]は家族に対してその心理的プロセスの援助者として優しく語りかけているが，家族としてももっと早い時期にセンター方式などを通じて認知症ケアを学んでいたらもっと上手く過ごせたのにと思うことがあるだろう。

このように考えると，認知症のどの段階からセンター方式を利用していくべきだろうか。医療機関でセンター方式を応用していくためにはどのようにすれば良いであろうか。早期診断を受けた直後からは介護保険サービスを利用しない場合も多いが，センター方式の持つ家族教育機能なども含めてこの時点からセンター方式を利用する意味はあると思われる。しかし，誰がどのようにセンター方式を運用するべきだろうか。介護保険サービスを使わない状態でもケアマネジャーが主治医と連携してセンター方式を通じた介入を開始できるような制度的改革を行うのが理想的かもしれない。

また，大きな問題点として本人への告知の問題も

高齢者医療における総合的機能評価(CGA)

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1　はじめに

高齢期には多くの疾病と老化による機能障害の複合により，生活機能が徐々に低下し，家庭及び社会生活の上で生活範囲が狭くなったり，介護が必要になることがある．高齢者の総合的機能評価(CGA；comprehensive geriatric assessment)は，そのような状態に対して多角的に評価を行い，医師，看護師，薬剤師，リハビリスタッフなどの多職種が専門性を生かして有効な援助法を提案し実施していく考え方である．介護保険にもその基本的な考え方が組み込まれ，その意義が理解されつつある．今後，日本では後期高齢者の増加が急速に進み，総合的機能評価の重要性が認識されていくものと思われる．

2　CGAの成り立ち

高齢者の総合的機能評価(CGA)という概念は，1930年代イギリスの女医マジョリー・ウォーレンがその当時老人病院に長期入院していた人たちを再評価した結果，適切なリハビリや社会的支援があればかなりの高齢者が退院できることを示したことから始まった．その後1980年代以降，アメリカでどのような対象者にどのような評価を行うことにより，どのような効果が得られるか検討が行われ，その生活機能障害を改善したり，入院日数を短縮する効果がある手法として広まってきた.[1] 現在，高齢化が急速に進む一方，臓器別医療で生活者としての人間像が奪われかねない日本の医療現場において，何が重要か見直す上でこの概念は不可欠である．

高齢者自身が医療サービスにどのようなことを望んでいるか英国で調査した結果がある．それによると優先順位として患者が選んだのは，①生活機能障害の改善，②QOLの改善，③介護者の負担軽減，④精神的ケアの改善，⑤高い活動性の順であり，効果ある医療が6番目，医療資源の活用は11番目，死亡率を減らすは12番目であった.[2] 今日，日本の医療現場では高齢者を診療することがかなりの比重を占めるが，医療提供者に同じ調査をしたら後者の3つが優先順位の上位にあがってくることはないだろうか？　高齢者の生活機能障害あるいはQOL(生活の質)を改善し，介護者の負担を軽減するためには医療提供者としてはどのようなアプローチが可能であろうか．高齢者はいうまでもなく，一人一人が複数の疾病を持ち，多くの種類の薬を内服している場合が多々見受けられる．そのうちどの病態を改善させることが最も本人の生活を守るために重要であるか，従来の医療モデルとは違う視点が必要となる．

3　ある事例をとおして

最初に，ある患者のことを例として紹介したい．82歳のその男性は，下痢や便秘を頻繁に訴え，さらに排尿障害や呼吸困難もあり，幾つもの診療科を受診するとともに時には夜間救急外来を受診していた．そして，その時々に対症療法的な処方がなされていた．ある時，高熱を

の日常生活動作能力の把握と見通しにより回復期リハビリテーション病棟及び退院後の訪問看護師のアレンジを行った．高齢者は若い人に比べて回復に時間がかかるので，その時々の状態に応じた急性期・回復期・慢性期の医療と，施設または在宅の福祉・介護サービスを段階的に選ぶことが大切である．[3a] 5つめとして，本人の生き甲斐・自負に視点を当てることにより本人の病気に取り組む前向きな姿勢を引き出した．この事例の場合，画家というやや特別な生活史があったが，本人の生き甲斐・自負をみいだすのに何も特別な職歴の持ち主である必要はない．「QOLを考えるとは，ある一面，他者のこころを知ろうという試みでもある．面倒なことに思えるか，強い印象を受けるか，こちらのスタンスで180度変わる」と高橋は指摘している．[3b]

4 CGAの意義と評価項目

以上，主に事例を通してCGAに必要な要素を見てきたが，CGAを行うことで具体的にどのような効果が期待されるであろうか．今までの報告では，入院回数の減少，入院日数の短縮，施設入所の減少，QOLの向上，服薬数の減少，ADL(activities of daily living；日常生活動作)の改善，死亡率の低下などが報告されている．[1b] CGAで評価する側面を改めて示すと表1のような身体的，精神・心理的，社会的側面が挙げられる．IADL(instrumental ADL；手段的ADL)やQOLなどは身体的でもあり精神・心理的でもあるが，便宜的に区切って提示した．ADLの評価シートであるバーセルインデックスや認知機能評価のための改訂版長谷川式簡易知能評価スケールのように，それぞれの評価項目に対して評価シートが存在する．[4] 使いやすさや多職種，他施設との交流を考えて適切なものを選び，使い慣れることが必要である．

5 CGAの対象と適用する場面

では，実際にどのような高齢者に対してCGAを行うことが有用であろうか．現在では後期高齢者とされる75歳以上の高齢者でも，一人で元気に社会生活を送っている人は少なくない．そのような高齢者すべてにCGAを行うことは，労力を考えると難しいだろう．CGAを行う最も良い適応は簡単な目安として「介護保険を使ってはどうか」とスタッフが考える高齢者であろう．介護保険の事前審査はまさにCGAである．つまり単一の，あるいは様々な複合的な要因によって何らかの手助けを行うことにより，その高齢者のADLやQOLの低下を防ぎ，向上することが期待される，あるいは援助なしでは生活を維持するのが難しいと思われる高齢者が最も良い対象となるであろう．そのような高齢者に対してどのような援助が必要であるか，多角的に検討する行為がCGAであり，そこからCGAを行う目的やゴールは自ずと見えて来るであろう．

CGAを適用する場面として介護保険関連の他に，病棟，外来，保健・予防，地域(フィール

表1 CGAで評価する項目

身体的側面
ADL(食事，入浴，トイレ動作，歩行，階段昇降など)
IADL(買い物，調理，洗濯，服薬管理，金銭管理，乗り物の利用など)
視聴覚，身体機能に影響を与えやすい合併症の有無，内服薬など
精神・心理的側面
認知機能(記憶，見当識，判断力など)
抑うつ度，意欲，QOL
社会的側面
居住形態(同居・独居，配偶者の有無など)，キーパーソン
経済状態，地域社会との交流，介護保険利用の有無
その他，介護負担度など

の目標に持っていくかも想像できる．また，在宅での介護がどの程度必要かの目安にもなる．それぞれの職種が更に詳しい評価を行う場合が多いが，共通の言語として簡潔なものがあることは有用である．

7 CGAの実施

CGAを有効に生かすためには，評価から出てくる個々の病態への理解と対応についての熟練が必要である．認知症，転倒・筋力低下，誤嚥・低栄養，失禁，薬剤の適切な使用などが重要だが，認知症についての知識と対応は特に重要である．厚生労働省の研究会報告「2015年の高齢者介護」にまとめられているように，今後の高齢者介護では寝たきりモデルから認知症モデルへの転換が進んでいくと予想され，CGAの実施にあたってもパラダイムの変化に気をつける必要がある．認知症の場合，本人の状態や家族の状況などの個別性に左右される要素が大きいので，ごく基本的なスクリーニングでも問題の所在を明らかにすることができるかもしれないが，十分な対応を行っていく上ではそれぞれの職種が更に深い評価と対応の技術を持つ必要がある．認知症の初期には内服の自己管理が難しくなってくる場合が多いので，薬剤師の協力も欠かすことができない．

その他の病態についても，例えば誤嚥・低栄養では言語聴覚士や耳鼻科医の協力による嚥下機能の評価やリハビリ，栄養士や薬剤師，消化器内科医の協力による食事，内服の工夫や胃ろうの検討などが欠かせない．失禁についても泌尿器科医や看護師の協力や技術，高齢者医療・介護に携わるすべてのスタッフが切迫性，溢流性，機能性，腹圧性失禁などの区別と基本的対応に関する知識を持つ必要がある．さらにこれらの実施が，高齢者やその家族の望みや本来持つ力を生かす形で行われるにはソーシャルワーク的視点が欠かせない．CGAはその名前(assessment)の通り評価が重要だが，評価の結果を確実に生かせる形での実施が行われることが，CGAを意義あるものとするといっても過言ではない．

8 結語にかえて

CGAを行うためには，常にそれぞれの専門職がいる必要はないし，30分～1時間程度(あるいはそれ以上)かかるアセスメントをすべて行う必要もない．しかし，それに関わるスタッフとしてはある期間，それぞれの専門職と共同作業を行って，お互いの専門性と果たす役割を知り，それぞれの評価項目の持つ意味を十分知ることが必要である．CGAは分かりにくく，とらえどころのない手法かもしれない．逆に言うとCGAを役立てるためには，明確な実体が見えにくいCGAの意義を理解し運営していく専門知識と目的意識が大切である．今後の高齢者医療のなかで，CGAが望ましい形で更に発展し浸透していくことを期待している．

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implications of the change of each of these two parameters in older subjects without cardiovascular disease, it can be hypothesized that exercise training improves functional cardiovascular capacity and vagal/sympathetic balance and that this effect is proportional to an improvement in lung ventilation. Future researches should investigate the significance of this correlation.

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ETIDRONATE AMELIORATES PAINFUL SOFT-TISSUE CALCIFICATION IN WERNER SYNDROME

To the Editor: Bisphosphonates, chemical compounds widely used as antiosteoporotic agents, were originally brought into clinical practice to treat ectopic calcification.[1] Their application for this purpose has been almost forgotten because the therapeutic dose may also affect normal bone formation. Here, we report that etidronate, a first-generation bisphosphonate, ameliorated soft-tissue calcification and improved performance in a patient with progeroid Werner syndrome without apparent adverse effects.

A 47-year-old woman visited our hospital because of intolerable pain in the left knuckle, bilateral elbows, and ankles. She had graying and loss of hair, peripheral soft tissue atrophy, a skin ulcer on the right ankle, marked insulin resistance, and a history of cataract at the age of 30. Werner syndrome was suspected; peripheral blood deoxyribonucleic acid (DNA) analysis confirmed homozygous type 4 mutations in the causative WRN helicase gene.[2]

Pain in the left knuckle was due to a hard subcutaneous nodule (Figure 1, left panel), which turned out to be an ectopic calcification (Figure 1, middle panel). Similar calcification was also found in the elbows and Achilles tendons; all of them coincided with the positions of pain. Her hands, elbows, and left ankle were free of ulcers. X-rays of the lumbar and thoracic spines showed no sign of osteoporosis. Serum calcium, phosphorus, alkaline phosphatase, and parathyroid hormone were in the normal range.

The patient could hardly clench her left fist or walk more than 1 m because of pain in the knuckle and ankles. Etidronate at a dose of 20 mg/kg per day was started orally in an attempt to suppress the ectopic calcification.

Clinical symptoms improved dramatically after 3 months of treatment. She was now able to walk for more than 6 m, was free of pain in the elbows, and felt remarkably less pain in her knuckle. The size of the nodule became smaller (Figure 1, right panel), indicating the effectiveness of etidronate in reversing calcification. No adverse effects were described at this point, but etidronate was stopped to avoid possible inhibition of bone formation.

Bisphosphonates, first synthesized in the 1860s, was originally used in industry to prevent scaling or precipitation of calcium carbonate.[1] Their biological effect of inhibiting ectopic calcification in vivo, as inspired by the structural similarity to inorganic pyrophosphate, was initially reported in 1968,[3] but clinical use of bisphosphonates for this purpose has not developed further, because they also interfere with mineralization of normal bone. Instead, they are now established as drugs against osteoporosis because of their property of preventing bone resorption when given at lower doses.

Werner syndrome, an autosomal recessively inherited progeroid disorder caused by homologous mutations in a RecQ family DNA helicase, often accompanies soft-tissue calcification for unknown reasons.[4,5] It can be asymptomatic but often results in severe pain and may promote skin ulcer formation. These symptoms limit patients' daily activity, threaten their quality of life, and facilitate development of overt diabetes mellitus due to inactivity on the base of insulin resistance.

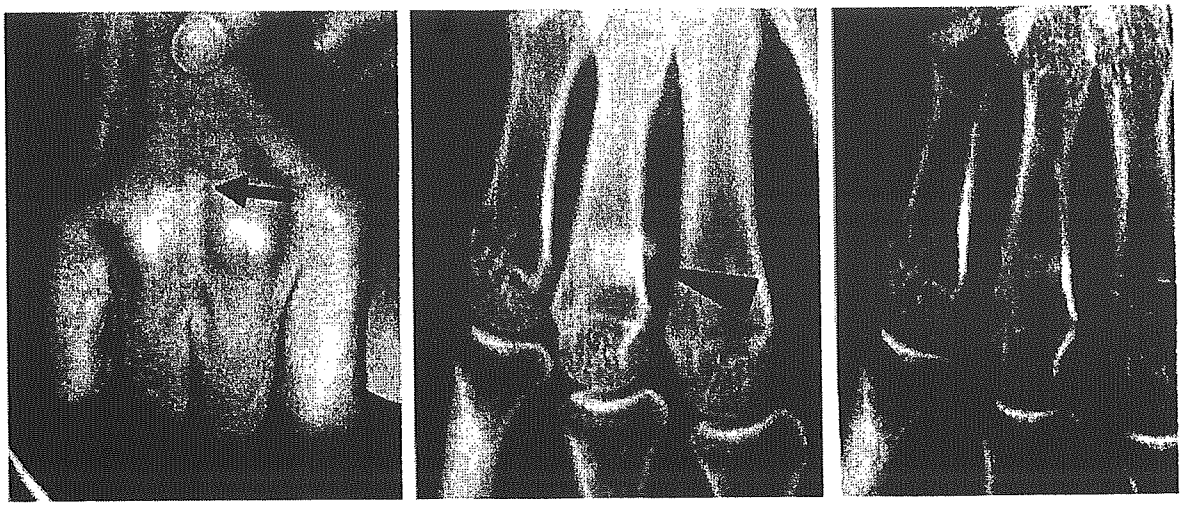

Figure 1. Left panel, subcutaneous nodule with pain (arrow). Radiographic examination before (middle panel) and after 3 months on etidronate (right panel). Arrowheads indicate the positions of nodular calcification.

Our present clinical experience demonstrates a novel therapeutic option for an otherwise incurable complication of Werner syndrome. Moreover, it rediscovers the usefulness of bisphosphonate for ectopic calcification.

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HYPOADIPONECTINEMIA IN BEDRIDDEN FEMALE PATIENTS YOUNGER THAN 75

To the Editor: Older people have several hormonal alterations, but the effect on the endocrine function of adipose tissue in older bedridden patients has not been fully elucidated. Adiponectin is a newly discovered antiinflammatory protein, secreted exclusively by adipocytes, that plays a protective role against atherosclerosis.[1] Hypoadiponectinemia plays a crucial role in atherosclerosis in men, but there have been no studies of plasma adiponectin in bedridden women. The aim of the present study was to estimate plasma adiponectin concentration in bedridden elderly female patients in comparison with age-matched healthy volunteers.

Seventy-four bedridden female patients admitted to geriatric wards and nursing homes in Osaka, Japan, and age-matched volunteers were studied. Clinical diagnoses were defined using detailed physical examination and routine biochemical analyses of blood and urine, as well as clinical tools including computed tomography. Their mean bedridden period ± standard deviation was 49.4 ± 37.4 months. All plasma analyses were performed on samples from fasting subjects. Adiponectin was measured using high-sensitive radioimmunoassay (Linco Research, St. Louis, MO). Bedridden subjects and healthy volunteers were divided into two groups: younger than 75 and aged 75 and older. All statistical analyses were performed using the SPSS (SPSS Inc., Chicago, IL). The statistical differences in the variables were compared using the Mann-Whitney *U* test, and the association between any two parameters was assessed using Spearman correlation.

Targeted Disruption of TGF-β–Smad3 Signaling Leads to Enhanced Neointimal Hyperplasia With Diminished Matrix Deposition in Response to Vascular Injury

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Abstract—The role of transforming growth factor (TGF)-β and its signal in atherogenesis is not fully understood. Here, we examined mice lacking Smad3, a major downstream mediator of TGF-β, to clarify the precise role of Smad3-dependent signaling in vascular response to injury. Femoral arteries were injured in wild-type and Smad3-null (null) male mice on C57Bl/6 background. Histopathological evaluation of the arteries 1 to 3 weeks after the injury revealed significant enhancement of neointimal hyperplasia in null compared with wild-type mice. Transplantation of null bone marrow to wild-type mice did not enhance neointimal thickening, suggesting that vascular cells in situ play a major role in the response. Null intima contained more proliferating smooth muscle cells (SMC) with less amount of collagen compared with wild-type intima. TGF-β caused significant inhibition of cellular proliferation in wild-type aortic SMC, whereas the growth of null SMC was only weakly inhibited by TGF-β in vitro, indicating a crucial role of Smad3 in the growth inhibitory function. On the other hand, Smad3-deficiency did not attenuate chemotaxis of SMC toward TGF-β. TGF-β increased transcript level of α2 type I collagen and tissue inhibitor of metalloproteinases-1, and suppressed expression and activity of matrix metalloproteinases in wild-type SMC. However, these effects of TGF-β were diminished in null SMC. Our findings altogether show that the loss of Smad3 pathway causes enhanced neointimal hyperplasia on injury through modulation of growth and matrix regulation in vascular SMC. These results indicate a vasculoprotective role of endogenous Smad3 in response to injury. (*Circ Res.* **2005;96:904-912.**)

Key Words: transforming growth factor-β ■ Smad3 ■ atherosclerosis ■ neointimal hyperplasia ■ smooth muscle cells

Transforming growth factor (TGF)-β is a prototypic member of the TGF-β superfamily that exerts a wide range of biological effects on various cell types.[1] Well described functions of TGF-β including growth inhibition, cell migration, differentiation, extracellular matrix production, and immunomodulation. Abnormality in TGF-β signaling may cause pathological conditions such as tumorigenesis, fibrotic disorders, and vascular diseases.[2] At present, however, the role of TGF-β and its signaling molecules in atherogenesis is not fully understood.

TGF-β is often regarded to have proatherosclerotic effect on arteries. For example, TGF-β expression is increased in human restenotic lesions as well as in neointimal hyperplasia after balloon injury in animals.[3] TGF-β facilitates extracellular matrix deposition by stimulating production of procollagen and fibronectin, downregulating the expression of proteases, and upregulating protease inhibitors, such as plasminogen activator inhibitor type I (PAI-I) and tissue inhibitor of metalloproteinase-1 (TIMP-1).[4–8] TGF-β transgene into vascular wall causes fibroproliferative intimal thickening in animal models in the presence or absence of vascular injury.[9,10] Moreover, TGF-β antagonism by antibody, soluble receptor, or ribozyme reduces constrictive remodeling after balloon injury in animals.[11–13]

On the other hand, considerable evidence implies antiatherosclerotic effects of TGF-β. TGF-β has been shown to inhibit proliferation and migration of vascular smooth muscle cells (SMCs) in vitro.[14,15] Inhibition of TGF-β signal systemically by use of neutralizing antibody and soluble TGF-β receptor type (TβR)-II or in T-cells by expressing a dominant-negative TβR-II results in an unstable plaque phenotype in mouse models of atherosclerosis.[16–18] SMCs

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obtained from human atherosclerotic plaques were shown to be defective in the TGF-β signal pathway and were resistant to TGF-β–mediated growth suppression and apoptosis.[19,20] Furthermore, low blood levels of active TGF-β were associated with severity of vascular disease in a manner consistent with an antiatherosclerotic effect of TGF-β.[21]

TGF-β elicits its effects via signaling through tetramerization of two different receptor serine/threonine kinases, TβR-I and TβR-II.[22,23] Activation of the receptors leads to phosphorylation of cytoplasmic signal transducers Smad2 and Smad3, classified as so-called receptor-activated Smads (R-Smad). The activated R-Smad heteroligomerizes with Smad4, a common mediator Smad, and the complex is transported to the nucleus where it regulates gene expression. In addition, pathways independent of Smads, which involve MAP kinases have also been described.[23] In mice lacking TGF-β signaling molecules, ie, TβR-I and TβR-II, Smad2 and Smad4 turned out to be embryonic lethal.[24–26] However, it was recently found that the mice null for Smad3 survive into adulthood.[27]

We undertook the present study examining Smad3-null mice in vivo and in vitro to elucidate the precise role of Smad3-dependent TGF-β signaling in the vascular response to injury.

Materials and Methods

Reagents

Reagents are described in an expanded Materials and Methods section in the online data supplement available at http://circres.ahajournals.org.

Mice

The generation of $Smad3^{ex8/ex8}$ null mice by homologous recombination was described previously.[27] See expanded Materials and Methods section for details.

Femoral Artery Injury

Mice femoral arteries were injured by use of photochemically-induced thrombosis method.[28] See expanded Materials and Methods section for details.

Histological Evaluation

Fixed femoral artery segments were embedded in paraffin and cut into 5-μm–thick serial sections. Six sections per one irradiated segment at 1-mm intervals were stained with hematoxylin and eosin. Neointima was defined as the region between the lumen and the internal elastic lamina. The media was defined as the region between the internal and external elastic lamina. The cross-sectional areas of intima and media were measured using NIH image version 1.62f (National Institutes of Health, USA). The intima-to-media (I/M) ratio was then calculated, and the mean I/M of all 6 sections per one irradiated segments was determined. The sections with intimal hyperplasia were also subjected to Masson's trichrome staining and immunohistochemistry. Masson's trichrome-positive intimal area was analyzed using Photoshop version 7.0 (Adobe). All the measurements were made in blinded manner.

Immunohistochemistry

Immunohistochemistry is described in the expanded Materials and Methods section.

Bone Marrow Transplantation

Bone marrow transplantation (BMT) was performed principally as described previously.[29] Briefly, bone marrow cell suspensions obtained from either Smad3-null or wild-type mice thigh bone were treated with ACK lysis buffer (0.155 mol/L ammonium chloride, 0.1 mol/L disodium EDTA, and 0.01 mol/L potassium bicarbonate) to lyse erythrocytes. The cells were intravenously injected to recipient Smad3-null or wild-type mice (1×10^6 per body) between the age of 6 and 9 weeks, 3 hours after lethal irradiation (8.5 Gy). Engraftment of the transferred bone marrow was confirmed by polymerase chain reaction (PCR) on peripheral blood DNA according to the protocol by Yang et al.[26] Femoral artery injury was performed 6 weeks after the bone marrow transfer.

Cell Culture

Mouse aortic SMCs were obtained and cultured as described by Ohmi et al[30] (see expanded Materials and Methods section). Experiments were performed on cells after 5 to 10 passages from the primary culture.

Immunocytochemistry

Immunocytochemical staining using anti–α-smooth muscle actin (SMA) and smooth muscle myosin (SMM) antibodies was performed as described by Hasegawa et al[31] with some modification (see expanded Materials and Methods section).

Immunoblotting

Immunoblotting was essentially performed as previously described[32] (see expanded Materials and Methods section).

Growth Inhibition Assay

Growth inhibition assay was performed as described by Datto et al[33] (see expanded Materials and Methods section).

Cell Migration Assay

SMC migration was evaluated by modified Boyden chamber method[34] (see expanded Materials and Methods section).

Real-Time Quantitative PCR

Real-time quantitative PCR is described in expanded Materials and Methods section.

Gelatin Zymography

Gelatin zymography is described in the expanded Materials and Methods section.

Statistical Analysis

Results were presented as mean±SEM. Statistical analyses used two-tailed, unpaired student *t* test.

Results

Mice Lacking Smad3 Show Enhanced Neointimal Hyperplasia in Response to Injury

To evaluate a role of Smad3 in the pathogenesis of neointimal hyperplasia, femoral arteries of wild-type (n=12) and Smad3-null (n=10) male mice were injured by use of the photochemically-induced thrombosis method.[28] Histopathological examination of the arteries 1 to 3 weeks after the injury revealed markedly enhanced neointimal thickening in Smad3-null mice compared with wild-type mice (Figure 1A and 1B). As shown in Figure 1C, mean I/M ratios evaluated at 1 and 3 weeks after the injury were significantly higher in Smad3-null arteries (0.193±0.034 at 1 week and 0.541±0.093 at 3 weeks) than those of wild-type arteries (0.059±0.018 at 1 week and 0.115±0.060 at 3 weeks, $P<0.01$ at each time point).

Immunohistochemical examination showed that both neointimal and medial cells were positive for α-SMA (Figure 2A and 2B) but negative for pan-leukocyte marker CD45 (Figure

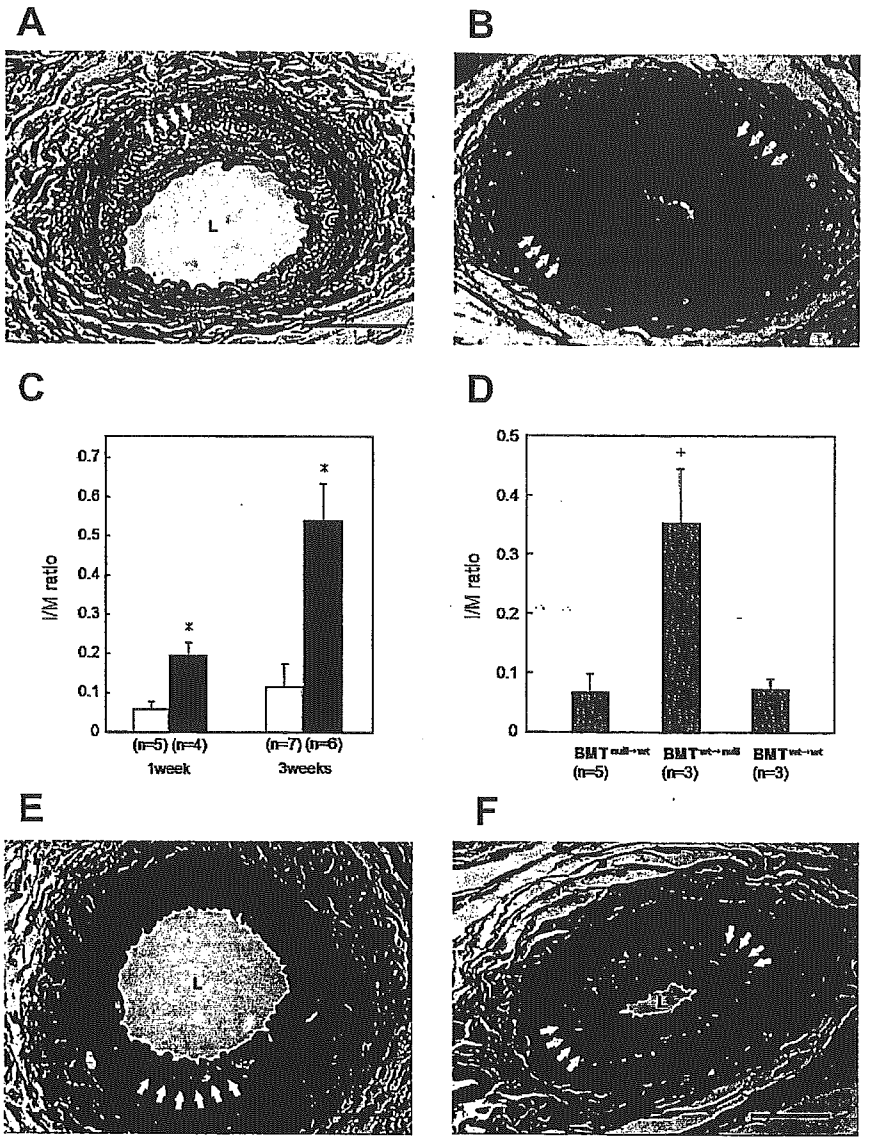

Figure 1. Neointimal thickening in injured femoral arteries of wild-type and Smad3-null mice. Photomicrographs showing representative cross sections of hematoxylin and eosin–stained femoral arteries from wild-type (A) and Smad3-null (B) and $BMT^{null\rightarrow wild}$ (E) and $BMT^{wild\rightarrow null}$ (F) mice 3 weeks after endothelial injury. L indicates vascular lumen. Arrows indicate the positions of the internal elastic lamina. Original magnification ×200; bar=50 μm. Intima-to-media (I/M) ratios at 1 and 3 weeks in wild-type and Smad3-null mice (C) and in $BMT^{null\rightarrow wild}$, $BMT^{wild\rightarrow null}$, and $BMT^{wild\rightarrow wild}$ at 3 weeks (D) were calculated from cross sectional areas morphometrically measured using an image analyzer. Open and closed columns indicate wild-type and Smad3-null mice, respectively. *$P<0.01$ compared with wild type at each time point; †$P<0.05$ compared with $BMT^{null\rightarrow wild}$.

2C and 2D), indicating that the intima was exclusively composed of SMCs. The same anti-CD45 antibody recognized leukocytes in vasa vasorum (Figure 2D) as well as lymphocytes in the mouse spleen (Figure 2E).

TGF-β is well known for its antiinflammatory effect.[1,2] To determine whether systemic inflammation due to Smad3 deficiency contributes to enhanced neointimal formation, we injured femoral artery of wild-type and Smad3-null mice after bone marrow transplantation (BMT). Lethally irradiated Smad3-null mice received 1×10^6 bone marrow cells from a wild-type mouse ($BMT^{wild\rightarrow null}$ mice). At the same time, irradiated wild-type mice were given bone marrow either from Smad3-null or wild-type mice ($BMT^{null\rightarrow wild}$ and $BMT^{wild\rightarrow wild}$ mice). Photochemical injury was performed 6 weeks after the bone marrow transfer, and the arterial cross section was analyzed 3 weeks later. As shown in Figure 1D, mean I/M ratio was significantly higher in $BMT^{wild\rightarrow null}$ arteries (0.353±0.091) than those of $BMT^{null\rightarrow wild}$ (0.067±0.031, $P=0.011$) or $BMT^{wild\rightarrow wild}$ (0.073±0.018, $P=0.039$) arteries. I/M ratios in $BMT^{wild\rightarrow null}$ and $BMT^{null\rightarrow wild}$ mice tended to be lower than those of Smad3-null and wild-type mice, respectively, presumably due to the effect of vascular irradiation.[35,36] Representative cross sections of $BMT^{null\rightarrow wild}$ and $BMT^{wild\rightarrow null}$ femoral arteries are shown in Figure 1E and 1F.

Smad3-Null Intima Is Rich in Proliferating Cells but Contains Low Amounts of Collagen Fibers

Intimal cell proliferation was assessed by immunohistochemical detection of proliferating cell nuclear antigen (PCNA) in the femoral artery sections 1 week after the injury (Figure 3A and 3B). The ratio of the PCNA-positive nuclei to total cell nuclei was higher by 1.8-fold in Smad3-null intima compared with wild-type intima (Figure 3C). The result shows an increased proliferative activity of SMCs in Smad3-null artery at the early stage after injury.

We next evaluated intimal cell density in hematoxylin and eosin–stained arterial sections 3 weeks after the injury. As shown in Figure 4A, the ratio of intimal cell number to total intimal area was 1.6-fold higher in Smad3-null artery

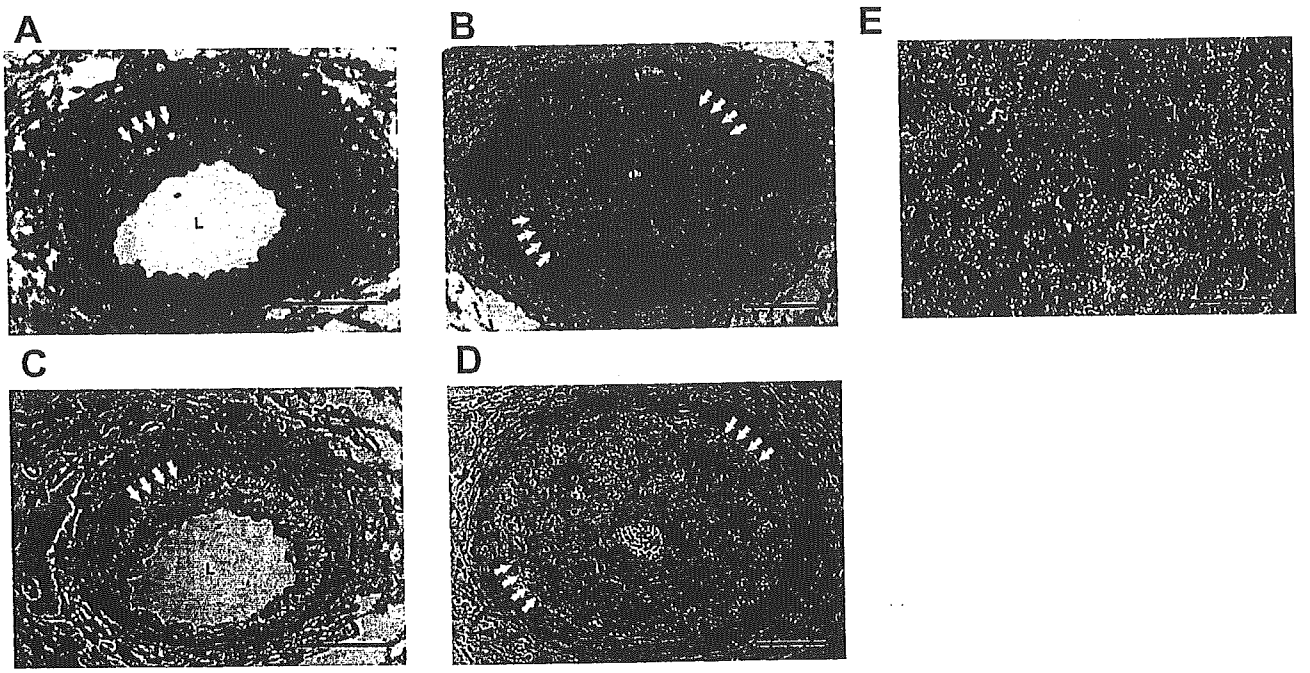

Figure 2. Immunohistochemical analysis of neointimal components. Cross sections of femoral arteries from wild-type (A and C) and Smad3-null (B and D) mice 3 weeks after endothelial injury and of mouse spleen (E). Immunostaining was performed using specific antibodies for α-SMA (A and B) and CD45 (C, D, and E). L indicates vascular lumen. Arrows indicate the positions of the internal elastic lamina. Arrowheads indicate the positions of representative CD45-positive leukocytes. Original magnification ×200; bar=50 μm.

(133±8.6) compared with wild-type artery (85.3±7.7, $P<0.01$), indicating higher cell density relative to extracellular area in Smad3-null intima. Because TGF-β/Smad3 signal is implicated in extracellular matrix (ECM) deposition, Masson's trichrome staining was also performed on a 3-week artery specimen to evaluate the amount of extracellular collagen fibers (Figures 4C and 4D). As summarized in Figure 4B, Smad3-null neointima showed 60% reduction in the ratio of Masson's trichrome-positive area to total intimal area compared with that of wild-type intima. These results suggest that Smad3 deficiency caused increased SMC number with less collagen deposition in neointima.

Growth Inhibition by TGF-β Is Attenuated in SMCs Lacking Smad3

To identify the mechanisms by which Smad3 deficiency caused exaggerated intimal hyperplasia, biological responses of the aortic SMCs obtained from wild-type and Smad3-null mice were examined in vitro. The cells were positive for both α-SMA and SMM (Figure 5A and 5B) as examined by immunocytochemistry. They also exhibited the classic "hills and valley" appearance, a feature characteristic of confluent cultured vascular SMCs. No morphological differences were observed between wild-type and Smad3-null SMCs (data not shown). It was confirmed by immunoblotting that SMCs derived from Smad3-null mice lacked expression of Smad3, whereas Smad2 level was similar in both cells (Figure 5C).

The SMCs were first tested for proliferation. As shown in Figure 6A, TGF-β dose-dependently inhibited FBS-stimulated DNA synthesis in wild-type SMCs with the maximal inhibition of 70% at 1 ng/mL and higher doses. In contrast, growth of Smad3-null SMCs was only weakly (<30%) inhibited by TGF-β. In addition, the basal growth rate of the null cells was ≈1.4-fold higher than that of the wild-type. Similar results were obtained for two additional cell lines of each genotype. The results firmly establish an essential role for Smad3 in TGF-β–mediated inhibition of cellular proliferation in vascular SMCs.

Smad3 Deficiency Does Not Attenuate TGF-β–Mediated Migratory Response in SMCs

The cells were next examined for migration, another function crucial to neointimal formation. Aschcroft et al[37] previously reported that Smad3-null monocytes and neutrophils were unable to migrate toward TGF-β, suggesting Smad3 is required for migration signal downstream of TGF-β. As shown in Figure 6B, Smad3-null SMCs dose-dependently migrated toward TGF-β at least to a similar extent as wild-type SMCs in a modified Boyden chamber assay. Moreover, Smad3-null cells showed a higher migratory capacity ($P<0.05$) than wild-type cells at 10 ng/mL TGF-β. The result suggests that Smad3-dependent signal is not essential for TGF-β–induced chemotaxis in murine vascular SMCs.

SMCs Require Smad3 for the Regulation of Type I Collagen, Matrix Metalloproteinases, and TIMP-1 by TGF-β

Previous studies suggested that migration of medial SMCs to intima involves extracellular matrix degradation.[38,39] Because TGF-β is implicated in extracellular matrix metabolism through transcriptional regulation of collagens, matrix metal-

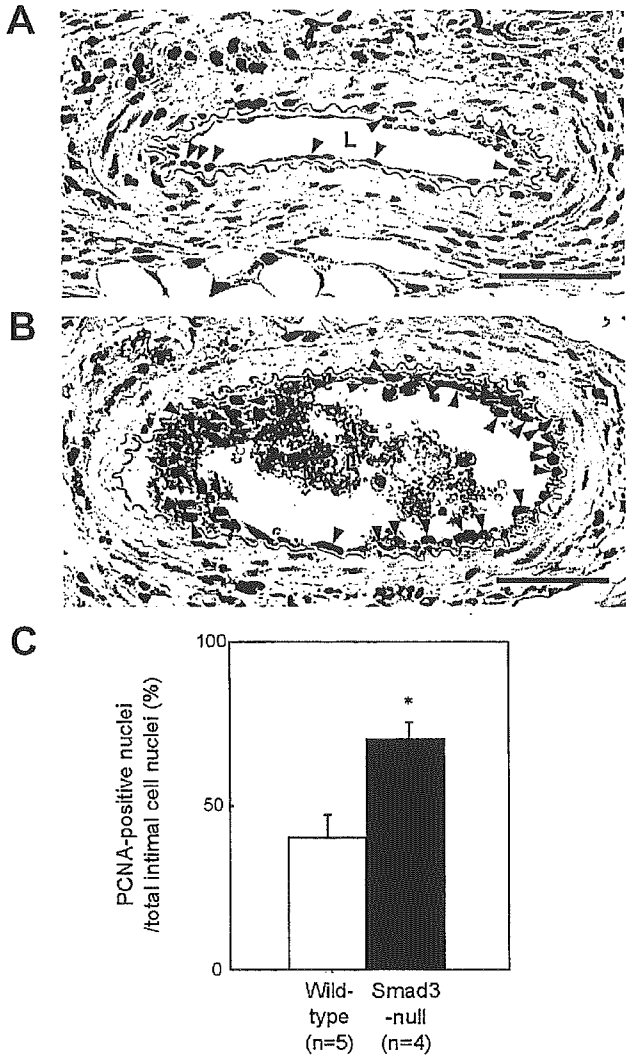

Figure 3. In vivo evaluation of cell proliferation in neointima. Representative anti-PCNA–stained cross sections of femoral arteries from wild-type (A) and Smad3-null (B) mice obtained 1 week after the injury. Arrowheads indicate PCNA-positive cells in intima. C, Ratios of PCNA-positive intimal cell number to total intimal cell number. L indicates vascular lumen. Original magnification ×200; bar=50 μm. *$P<0.05$ compared with the wild type.

loproteinases (MMPs), and TIMP-1,[7,8] we examined the ability of TGF-β to regulate mRNA expression of these components in wild-type and Smad3-null SMC. Transcript levels of COL1A2, membrane-type matrix metalloproteinase 1 (MT1-MMP), and TIMP-1 were evaluated by real-time quantitative PCR. As shown in Figure 7A, TGF-β time-dependently upregulated mRNA level of COL1A2 in wild-type SMCs with a maximal increase of 3-fold. Induction of COL1A2 by TGF-β was significantly less in Smad3-null SMCs compared with wild-type cells at all time points. TGF-β suppressed mRNA expression of MT1-MMP, an activator of pro–MMP-2,[40] to 64% of the basal level in wild-type SMCs (Figure 7B). However, MT1-MMP level was not affected by TGF-β in Smad3-null SMCs. Moreover, TGF-β increased TIMP-1 expression by 5-fold over the basal level in wild-type SMCs (Figure 7C), whereas no significant induction was observed in Smad3-null SMCs. Finally, the effect of TGF-β on MMP activity in SMC culture media was examined by gelatin zymography (Figure 7D). The basal gelatinolytic activity of MMP-2 in a serum-free conditioned media was similar for wild-type and Smad3-null SMCs. TGF-β time-dependently suppressed MMP-2 activity in wild-type cells with the maximal suppression of 29% at 24 hours, but it did not show significant effect in Smad3-null SMCs. These results suggest that Smad3 plays an essential role in TGF-β–mediated regulation of type I collagen, MMPs, and TIMP-1 in vascular SMCs.

Discussion

We report six novel findings in this article. First, mice lacking Smad3 showed a significant enhancement of neointimal hyperplasia on endothelial injury compared with corresponding wild-type mice. Second, neointima of Smad3-null mouse after injury contained a larger number of PCNA-positive cells compared with wild-type, indicating an increased proliferative activity of Smad3-null SMCs in vivo. Third, Smad3-null neointima showed higher cell density with reduced collagen area. Fourth, TGF-β–induced growth inhibition was diminished in Smad3-null SMCs in vitro. Fifth, Smad3-null SMCs retained migratory activity toward TGF-β. And finally, Smad3-null SMCs were impaired in induction of type I collagen and TIMP-1 as well as in suppression of MMPs by TGF-β. These results confirm a regulatory role of endogenous Smad3 in vascular remodeling in response to injury.

Enhanced neointimal hyperplasia in Smad3-null mice (Figure 1) lend support to previous reports describing the association of low TGF-β activity either at the ligand or receptor levels with intimal lesion formation. Grainger et al[41] showed that transgenic expression of apolipoprotein(a) promoted SMC proliferation and subsequent development of early vascular lesions by inhibiting proteolytic activation of TGF-β. Conversely, treatment with the antiestrogen tamoxifen increased serum TGF-β_1 levels and suppressed the formation of aortic lesions in mice[42]; a similar effect was also observed in human subjects.[43] McCaffrey et al[19] reported that reduced TβR-II activity due to genomic mutations led to SMC expansion in human atherosclerosis. Moreover, inhibition of TGF-β by use of a soluble type II receptor or a neutralizing antibody accelerated atherosclerosis and induced an unstable plaque phenotype in apoE-deficient mice.[17,18] And our present findings, for the first time, demonstrate a direct evidence that attenuation of TGF-β signal at the postreceptor level results in enhanced neointimal formation on injury.

Increased PCNA-positive intimal cells in vivo (Figure 3) and defect in TGF-β–induced growth suppression in vitro (Figure 6A) suggest that increased proliferative activity of SMCs contributes to the prominent neointimal formation in Smad3-null mice. Importance of Smad3 in TGF-β–mediated growth inhibition has well been described in other cell types such as αCD-stimulated primary splenocytes and embryonic fibroblasts.[33] Our results verify that Smad3, also in vascular SMCs, plays a major role in growth inhibitory function of

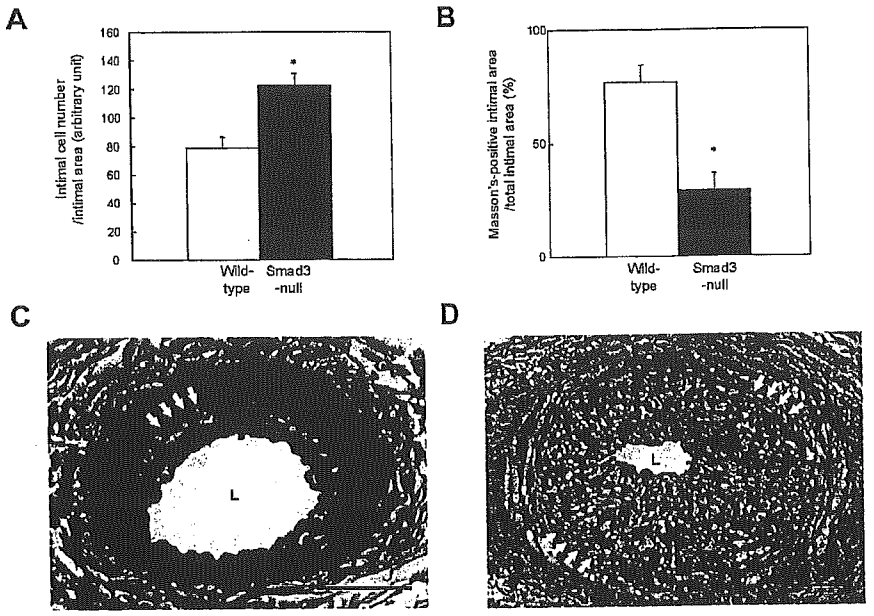

Figure 4. Evaluation of cell density and matrix deposition in neointima. A, Ratios of intimal cell number to total intimal area evaluated on hematoxylin and eosin–stained femoral arterial sections from wild-type (n=7) and Smad3-null (n=6) mice obtained 3 weeks after the injury. B, Ratios of Masson's trichrome–positive intimal area to total intimal area in femoral arterial sections from wild-type (n=7) and Smad3-null (n=6) mice 3 weeks after the injury. C and D, Photomicrographs showing the representative Masson's trichrome–stained sections of wild-type (C) and Smad3-null (D) femoral arteries. Arrows indicate the positions of the internal elastic lamina. L indicates vascular lumen. Original magnification ×200; bar=50 μm. *$P<0.01$ compared with the wild type.

TGF-β. It is to be noted that lack of Smad3 did not eliminate TGF-β–induced growth suppression in SMCs (Figure 6A). The residual growth inhibitory activity is likely to depend on another mediator downstream of TGF-β receptors, possibly Smad2.

Ashcroft et al[37] reported that Smad3 is required for TGF-β–induced migration of monocytes, leukocytes, and keratinocytes. Unexpectedly, Smad3-null SMCs were able to migrate toward TGF-β (Figure 6B). The finding suggests that, in contrast to the growth inhibitory function, Smad3-dependent signal is not essential for chemotaxis by TGF-β in murine vascular SMCs. It is therefore likely that the ability of medial SMCs to migrate into intima is preserved in Smad3-null arteries. The signaling pathway responsible for TGF-β–induced SMC motility remains to be elucidated.

TGF-β is known as a potent inducer of ECM deposition. It has been demonstrated that overexpression and intravenous administration of TGF-β caused arterial intimal thickening largely consisted of increased ECM.[10,44] TGF-β exerts fibrogenic activity through enhancement of ECM synthesis as well as inhibition of ECM degradation by downregulating MMP expression and upregulating MMP inhibitors.[6–8] Previous studies, mainly performed on dermal fibroblasts, showed that TGF-β–mediated regulation of many ECM-related genes, such as type I, III, V, and VI collagens, TIMP-1 and MMP-1 was Smad3-dependent.[45–47] In this study, we reported that Smad3-null neointima was rich in SMCs with relatively less matrix-deposition compared with wild-type intima, as evaluated by intimal cell density and Masson's trichrome staining (Figure 4), confirming a crucial role of Smad3-dependent signals in vascular ECM regulation. Moreover, TGF-β was unable to enhance mRNA expression of COL1A2 and TIMP-1 or suppress MT1-MMP expression in Smad3-null SMCs (Figure 7), establishing Smad3-dependency of these genes in vascular SMCs. Regulation of MMP-2 or gelatinase also seems to depend on Smad3-pathway in SMCs, because TGF-β attenuated MMP-2 activity in the culture media of wild-type but not in Smad3-null SMCs. Because degradation of matrix scaffold by MMPs enables cell movement and general tissue reorganization,[38,39] inability of TGF-β to suppress MMPs in Smad3-null SMCs may facilitate cell migration from media to intima in vivo.[48] Our in vitro finding that Smad3-null SMCs show a higher migration than wild-type at 10 ng/mL TGF-β (Figure 6B) may support this idea. MMP activity uninhibited by TGF-β as well as decreased matrix deposition might also have contributed to enhancement of intimal thickening in Smad3-null mice.

There have been reports on injury models suggesting that TGF-β promotes intimal thickening.[3,9–13,49] The present result that Smad3 deficiency accelerates intimal response to injury appears inconsistent with these results. However, we do not think that our findings contradict other reports on TGF-β transgene or antagonism. Our model differs from any other previous models in the point it specifically lacks Smad3 signal but not other TGF-β signal components, eg, Smad2 and MAP kinases. Smad3 not only transduces signal downstream of TGF-β, but also plays a major role in signaling of activins,[22,23] other members of the TGF-β superfamily. Activin A is expressed in atherosclerotic lesion[50] and promotes the contractile or nonproliferative phenotype of SMCs,[51] playing a role in stabilization of atherosclerotic plaque. Adenovirus-mediated overexpression of activin A suppresses neointimal formation.[51] Although we have not examined the involvement of activin A in the present study, it is assumable that the defect in activin A signal in addition to TGF-β accounts for the drastic neointimal hyperplasia in Smad3-null mice. It is of interest to determine whether specific activation of Smad3 in arterial SMCs in vivo attenuates neointimal hyperplasia. As another possibility, proinflammatory status caused by systemic Smad3 deficiency[27] might have influenced neointimal response. Although our BMT results (Figure 2D through 2F) show that the degree of intimal hyper-

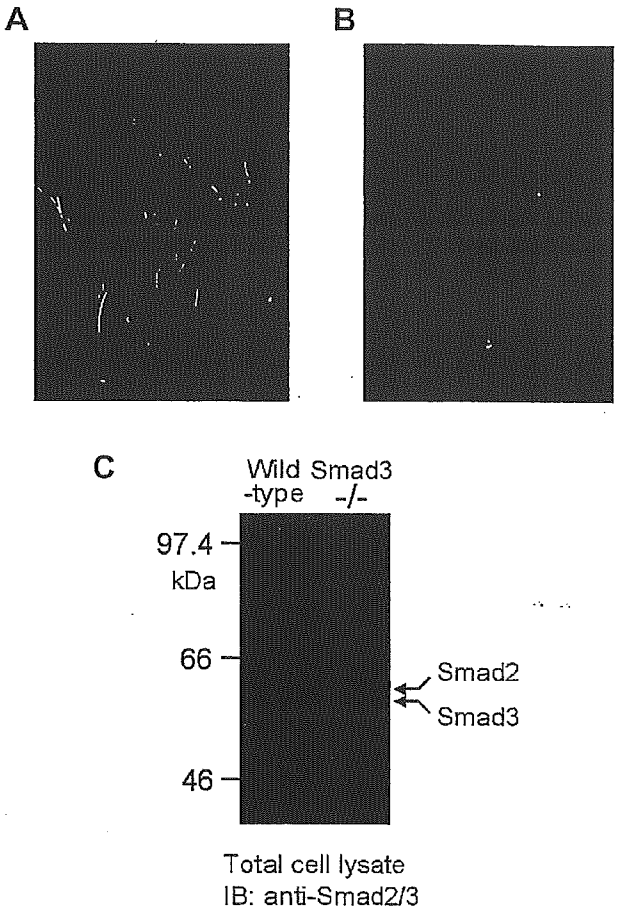

Figure 5. Characterization of cultured mice aortic SMCs. SMCs enzymatically isolated from the aorta of wild-type mice were immunocytochemically stained using anti-SMA (A, green) and anti-SMM (B, red) antibodies, counterstained with DAPI (blue, for nuclei), and subjected to fluorescent microscopy. Original magnification ×200. C, Total cell lysates of wild-type and Smad3-null SMCs were analyzed by SDS-PAGE and subjected to immunoblotting with an anti-Smad2/3 antibody. Migration positions of Smad2 and Smad3 are indicated.

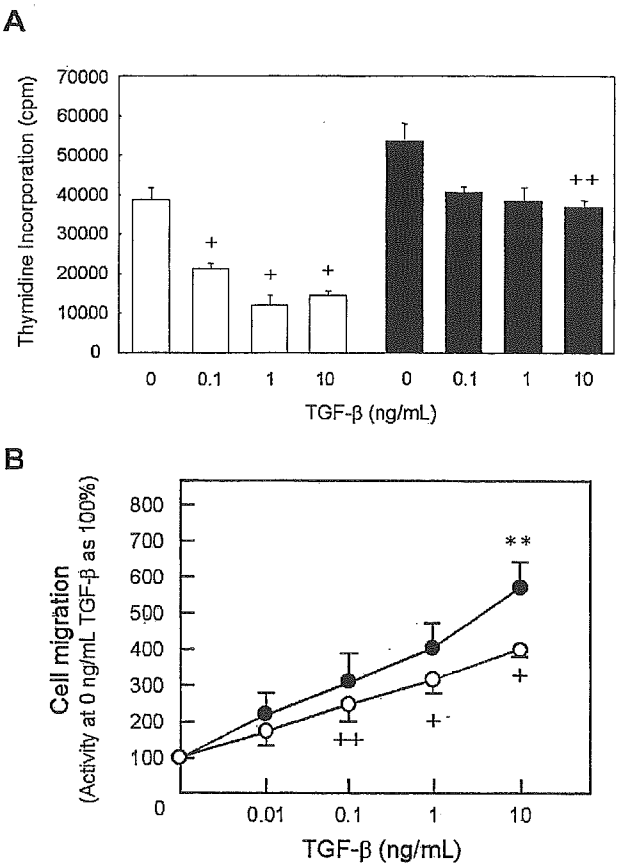

Figure 6. TGF-β–induced growth inhibition and migration of wild-type and Smad3-null SMCs. A, Wild-type (open columns) and Smad3-null (closed columns) SMCs were assayed for TGF-β–induced growth inhibition using ^{3}H-thymidine incorporation. Data are expressed as the means of three separate experiments, each performed in quadruplicate. $+P<0.01$, $++P<0.05$, compared with the value of 0 ng/mL TGF-β. B, Migration of wild-type (open circles) and Smad3-null (closed circles) SMCs toward various doses of TGF-β was measured by use of modified Boyden chamber method. Data represent the percentage of cell numbers relative to those in the absence of TGF-β and are expressed as the means of 5 separate experiments, each performed in triplicate. $+P<0.01$, $++P<0.05$, compared with the value of 0 ng/mL TGF-β. $**P<0.05$, compared with the value of wild-type at 10 ng/mL TGF-β.

plasia mainly depends on the origin of blood vessels and not of bone marrow cells, further investigation is needed to elucidate the entire role of inflammation in Smad3-null vascular response.

Finally, overactivation of TGF-β–Smad3 pathway is implicated in various fibrotic diseases involving organs such as skin, lung, liver, and kidney. Molecular agents that block Smad3-dependent TGF-β signal are anticipated as an ideal therapeutic option for these disorders.[46] However, our present results lead us to surmise that systemic suppression of Smad3 signaling can cause undesirable effects in the arteries by facilitating proliferative intimal lesions. Therefore, selective drug-delivery to the affected organs as well as careful monitoring of possible vascular lesions should be considered on clinical application of Smad3 inhibitors for fibrotic diseases.

In conclusion, mice lacking Smad3 developed marked neointimal hyperplasia on injury accompanying modulation of growth and matrix regulation in vascular SMCs. This study documents direct evidence and novel information on the functional significance: a vasculoprotective role of Smad3-dependent TGF-β signaling in response to injury.

Acknowledgments

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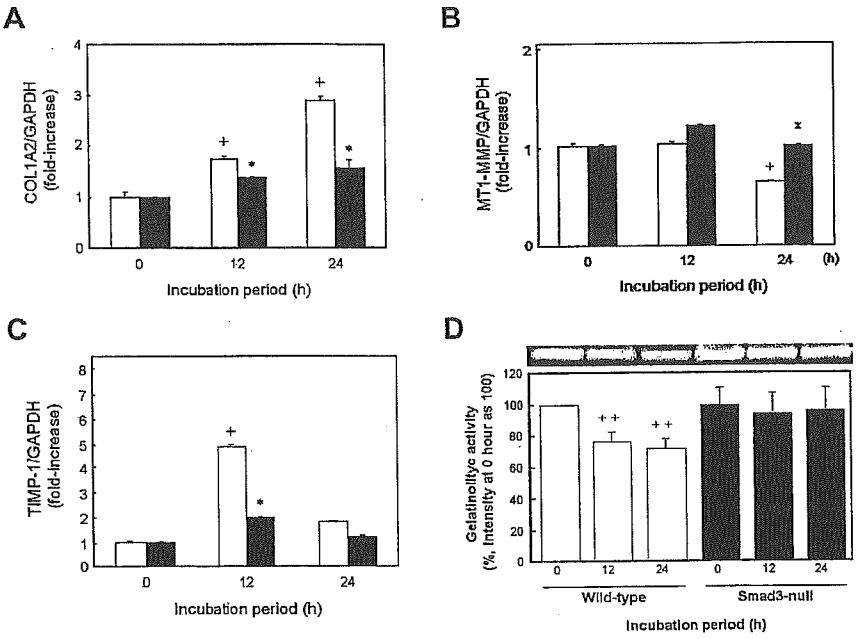

Figure 7. Effect of TGF-β on expression of type I collagen, MMPs, and TIMP-1 in wild-type and Smad3-null SMCs. Transcript levels of COL1A2 (A), MT1-MMP (B), and TIMP-1 (C) in wild-type and Smad3-null SMCs treated with TGF-β. Wild-type (open columns) and Smad3-null (closed columns) SMC were incubated with 10 ng/mL TGF-β for the indicated periods, the total RNA was isolated and used for cDNA synthesis. Quantitative real-time PCR was performed using the SYBR Green PCR Master Mix and analyzed on an ABI PRISM 7000 Sequence Detector System. Data were calculated relative to the value for the cells without TGF-β and are expressed as the means of 3 separate experiments, each performed in triplicate. $+P<0.01$, compared with the value of 0 hour; $*P<0.01$, compared with the wild type at the same time point. D, MMP-2 gelatinolytic activity in the culture media of wild-type and Smad3-null SMCs treated with TGF-β. Culture media of SMCs incubated with 10 ng/mL TGF-β for the indicated periods was analyzed by gelatin zymogram. Proteolytic degradation of gelatin by MMP was visualized as a translucent band on the dark background. Graph shows the gelatinolytic activity, evaluated by densitometrical scanning of the bands, relative to those of wild-type SMCs at 0 hour. Data were expressed as the means of 4 separate experiments. $++P<0.05$, compared with the value of 0 hour.

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