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代表・分担研究報告書④

腹腔外発生デスモイド腫瘍患者の実態把握および診療ガイドライン確立に向けた研究

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研究要旨　腹腔外デスモイド腫瘍患者に対するメロキシカム治療の成績とβ-カテニン遺伝子（CTNNB1）変異型との関連を明らかにすることを目的とし、生検組織からDNAを抽出し、CTNNB1エクソン3の変異型を特定し、各臨床因子および変異型とメロキシカムの治療成績との関連を解析した。33症例中21例（64%）でCTNNB1エクソン3に変異を認め、16例(49%)はT41A変異であり、4例（12%）はS45F、1例ではS45P変異を有していた。変異型とメロキシカム治療への反応性との間に関連を認め（P=0.053）、特にS45F変異を有する4例はすべてメロキシカムへの反応性がPDであった（P=0.017）。CTNNB1遺伝子変異型を特定することで、メロキシカム治療の反応性を予測できる可能性が示された。

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A. 研究目的

腹腔外発生デスモイド腫瘍に対する治療方針は広範切除による手術が主であったが、高い再発率と術後の機能低下から、保存的治療法やwait & see（経過観察のみ）を選択する施設が増えている。しかし、治療方針を決定するために明確な基準はなく、治療施設間での治療方針は大きく異なっている。各種治療法の臨床成績に関与する因子を同定し、治療前に評価することが可能であれば、デスモイド腫瘍患者に治療方針を明確に説明することができ、全国共通の治療ガイドラインを確立することが可能となる。腹腔外発生デスモイド腫瘍の多くはβ-カテニン遺伝子（CTNNB1）の変異が原因となって発症する。またデスモイド腫瘍にお

ける CTNNB1 変異型はいくつか報告されており、最近 CTNNB1 変異型と手術治療成績との関連が報告されている。しかし、保存的治療法と CTNNB1 変異型間との関連性については報告がない。本研究では、腹腔外デスモイド腫瘍における CTNNB1 変異の有無とその型を特定し、変異型とメロキシカムによる保存治療の臨床成績との関連を解析することを目的とした。

B. 研究方法

2003 年以降、41 例の腹腔外デスモイド腫瘍患者に対して、前向きに COX-2 阻害剤であるメロキシカム治療を実施した。経過期間の短い症例などを除外して、33 例の前治療を実施していない患者を対象とした。メロキシカム治療の効果は定期的に撮像する MRI あるいは CT 画像により RECIST 基準に従い、CR（腫瘍消失）、PR（腫瘍縮小）、SD（腫瘍不変）、PD（腫瘍増大）に評価・分類した。全例生検を実施し、凍結保存あるいはホルマリン固定後パラフィン包埋した標本から DNA を抽出した。CTNNB1 のエクソン 3 を標的として、PCR にて増幅し、その産物を用いてダイレクトシークエンス法により DNA 配列を決定した。決定したシークエンスは NCBI-BLAST のデータベースと比較し、変異部位と型を特定した。CTNNB1 変異型と各臨床因子（性、年齢、腫瘍サイズ、発生部位）との関連を解析した。また変異型とβ-カテニン核内染色性との関連を調査した。RECIST によるメロキシカム治療成績と変異型との関連を解析した。

（倫理面への配慮）

患者の各種臨床因子、治療成績に関わる後ろ向き調査については、個人情報の取り扱いに十分注意し、臨床研究に関する倫理指針（平成20年7月31日全部改正）に準じ、また、遺伝子変異型解析についてはヒトゲノム・遺伝子解析研究に関する倫理指針（平成20年12月1日一部改正）に準じ、名古屋大学医学系研究科倫理委員会の承認を得た上で、研究参加者の書面での同意を得た上で行った。

C. 研究結果

33 症例の腹腔外デスモイド腫瘍は、腹壁発生 7 例、頚部 5 例、背部と前腕が各 4 例、胸壁と大腿各 3 例、肩 2 例、その他 5 例であった。放射線治療を含めて前治療を実施した症例はなく、経過観察期間の中央値は 36.6 ヶ月であった。RECIST では、CR1 例、PR7 例、SD12 例、PD13 例であり、PD13 例中、3 例は手術を実施し、8 例には抗がん剤治療を実施した。メロキシカム治療成績良好群と不良群の間に、性(P=0.46)、年齢(P=0.34)、腫瘍サイズ（P=0.63)、発生部位（P=0.22）に有意差を認めなかった。CTNNB1 のエクソン 3 の変異は 33 症例中 21 例(64%)に認め、コドン 41 と 45 だけに存在した。16 例(49%)は T41A 変異であり、4 例（12%）は S45F、1 例では S45P 変異を有していた。CTNNB1 変異型と性（P=0.67)、年齢（P=0.57)、腫瘍サイズ（P=0.47)、発生部位（P=0.23）の間に関連を認めなかった。

変異型とメロキシカム治療への反応性との間に関連を認め（P=0.053)、特に S45F 変異を有する 4 例はすべて反応性が PD であった（P=0.017)。またβ-カテニンの核内染色強陽性と S45F 変異型は有意に関連していた（P=0.035)。

D. 考察

Wnt-β-カテニン経路の異常がデスモイド腫瘍の病態と関連している。CTNNB1 変異の中で、T41A の頻度が最も高いとされる過去の報告は本研究の結果と合致していた。CTNNB1 変異型と手術治療後の再発率との関連が報告されている。S45F 変異型症例は有意に高く再発するとの報告があるが、同様の関連を認めなかったとする報告もあり、まだ統一した見解が得られていない。本研究では S45F 変異型 4 症例はすべてメロキシカム治療に抵抗性であり、変異型によりメロキシカム治療を回避すべき症例があることを示唆している。我々はβ-カテニンの核内の免疫組織染色性がメロキシカムの治療成績と関連することを報告したが、遺伝子変異型特定のほうが安定した情報を提供することが可能である。今後、症例数を増やす必要があるが、CTNNB1 遺伝子変異型特定により、メロキシカム治療の奏功性に関して、デスモイド腫瘍患者・医療者に治療法選択に関する情報を提供できると考える。

E. 結論

前向きにメロキシカム治療を実施した 33 例の腹腔外デスモイド腫瘍患者に対して、生検組織から抽出した DNA により CTNNB1 のエクソン 3 の遺伝子変異の有無・型を同定し、各種臨床因子およびメロキシカムの治療成績との関連を解析した。S45F を有する症例はメロキシカム治療に対する反応性が有意に不良であり、遺伝子変異型解析により治療前にメロキシカムへの反応性を予測できる可能性を示した。

F. 研究発表

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腫瘍径の大きなデスモイド腫瘍に対する治療アルゴリズム
第 47 回日本整形外科学会骨・軟部腫瘍学術集会 2014.7.17-18　大阪

濱田俊介, 浦川浩, 新井英介, 小澤英史, 二村尚久, 生田国大, 筑紫聡, 石黒直樹, 西田佳弘
デスモイド腫瘍に対するメロキシカム治療の効果予測　CTNNB1(β-catenin)遺伝子変異による
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濱田俊介, 浦川浩, 小澤英史, 筑紫聡, 石黒直樹, 西田佳弘
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第 123 回中部日本整形外科災害外科学会・学術集会　2014.10.3-4　名古屋

G. 知的財産権の出願・登録状況
なし

厚生労働科学研究費補助金　難治性疾患等政策研究事業（難治性疾患政策研究事業）
代表・分担研究報告書⑤

腹腔外発生デスモイド腫瘍患者の実態把握および診療ガイドライン確立に向けた研究

研究代表者　西田 佳弘　名古屋大学大学院医学系研究科整形外科　准教授

研究要旨　腹腔外デスモイド腫瘍で他治療に抵抗性を示す患者に対して低用量メソトレキセート（MTX）とビンブラスチン（VBL）治療の有効性と安全性を明らかにし、治療成績と各種臨床因子およびβ-カテニン遺伝子（CTNNB1）変異型との関連を解析することを目的とした。各臨床因子および CTNNB1 変異型と MTX+VBL の治療成績との関連を解析した。PR6 例、SD8 例、PD1 例であり、各臨床因子および CTNNB1 変異型のいずれも MTX+VBL 治療効果と有意な関連を認めなかった。1 例で間質性肺炎を生じ、治療を中止した。2 週間間隔の投与では副作用の多くは grade 1 であった。2 週間間隔の低用量 MTX+VBL 治療は副作用が少なく、長期間継続可能な治療法で、S45F 変異型腫瘍に対しても有効性が期待できる治療である。

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A. 研究目的

腹腔外発生デスモイド腫瘍に対する治療の中心は手術による広範切除であったが、高い再発率から保存的治療を選択する施設が増えている。また発生部位によっては切除が困難なデスモイド腫瘍があり、保存治療を選択せざるを得ない症例がある。これらの手術治療困難症例、NSAID などの薬物治療に抵抗性を示す症例に対して、抗がん剤治療、特に低用量のメソトレキセート（MTX）とビンブラスチン（VBL）の併用療法に関する報告がある。しかし、どのようなデスモイド腫瘍患者に MTX+VBL の適応があるのか、また治療効果の予測因子に関する報告はほとんどない。MTX+VBL 療法の適応、効果予測に関する知見は稀少疾患であるデスモイド腫瘍に対する全国共通の治療ガイドラインを示すためには重要な情報である。腹腔外発生デスモイド腫瘍の多くはβ-カテニン遺伝子（CTNNB1）の変異が原因となって発症する。この変異の有無、型が MTX+VBL 療法の治療成績に関与する可能性

がある。本研究では、日本人の腹腔外デスモイド腫瘍に対する低用量 MTX+VBL 療法の効果と安全性を明らかにし、治療成績に影響する因子として、特に CTNNB1 変異型に着目して解析することを目的とした。

B. 研究方法

2003 年以降、腹腔外デスモイド腫瘍患者に対して前向きにメロキシカム治療を実施した 68 例中 24 例が治療抵抗性を示し、その中で切除困難症例 14 例に低用量 MTX (30mg/M2)+VBL(6mg/M2)療法を実施した。またメロキシカム治療を実施していない小児患者 1 例に対しても本治療を実施し、計 15 例を対象とした。投与間隔は基本的に 2 週間に 1 サイクルとし、治療効果は定期的に撮像する MRI あるいは CT にて RECIST 基準に従って評価 (CR:腫瘍消失、PR:腫瘍縮小、SD:腫瘍不変、PD:腫瘍増大) した。無増大生存率を Kaplan-Meier 法にて算出した。CR と PR を responder 群、SD と PD を nonresponder 群として、各種臨床因子 (性別、年齢、初発・再発、腫瘍サイズ、治療期間、治療回数) と MTX+VBL 療法の有効性との関連を解析した。凍結保存あるいはホルマリン固定後パラフィン包埋した標本から DNA を抽出した。CTNNB1 のエクソン 3 を標的としてプライマーを作成し、PCR にて増幅し、その産物を用いてダイレクトシークエンス法により DNA 配列を決定した。決定したシークエンスは NCBI-BLAST のデータベースと比較し、変異部位と型を特定した。CTNNB1 変異型と MTX+VBL 療法に対する有効性との関連を解析した。副作用は National Cancer Institute Common Terminology Criteria (NCI-CTCAE v4.0) の基準に従った。

(倫理面への配慮)

患者の各種臨床因子、治療成績に関わる後ろ向き調査については、個人情報の取り扱いに十分注意し、臨床研究に関する倫理指針 (平成20年7月31日全部改正) に準じ、また、遺伝子変異型解析についてはヒトゲノム・遺伝子解析研究に関する倫理指針(平成20年12月1日一部改正) に準じ、名古屋大学医学系研究科倫理委員会の承認を得た上で、研究参加者の書面での同意を得た上で行った。

C. 研究結果

全例日本人患者で、家族性大腸腺腫症を有する患者は含まれなかった。15 例中、13 例が体幹部発生、2 例が四肢発生、6 人が男性で、9 人が女性であり、全例放射線治療歴はなかった。MTX+VBL 治療期間と治療回数の中央値はそれぞれ 16 ヶ月、25 サイクルであった。RECIST による評価で、PR6 例、SD8 例、PD1 例であり、PD1 例についてはドキソルビシンとダカルバジンによる併用化学療法にて著明に腫瘍は縮小した。年齢 (P=0.75)、性(P=0.91)、初発・再発(P=0.19)、腫瘍サイズ (P=0.75)、治療期間 (P=0.45)、治療回数 (P=0.93)、CTNNB1 変異型 (P=0.52) のいずれも治療効果と有意な関連を認めなかった。S45F 変異型がメロキシカム治療の成績不良と有意に関連するとの結果を得ているため、変異型を S45F とそれ以外の 2 群に分けて解析したところ、MTX+VBL の治療効果と関連を認めなかった (P=1)。PR あるいは SD を示した 5 例の患者で MTX+VBL 治療を中止したが、そのうち 4 例で腫瘍の再増大を認めなかった。腫瘍の再増大を認めた

1 例は S45F 型であった。副作用については、1 例で間質性肺炎を生じ、治療を中止した。毎週投与した患者については grade 4 の好中球減少を 1 例に認め、grade 3 の GOT あるいは GPT 上昇を 3 例に認めた。2 週間間隔の投与では多くは grade 1 の副作用であった。

D. 考察

本研究結果は、低用量の MTX+VBL 化学療法がアジア人に対して実施可能であることを初めて明らかにした。また 4 例では 2 年以上にわたって投与を継続していることから、長期間の治療が可能であることを示している。デスモイド患者に対する MTX+VBL 治療の過去の報告と比較して、有効性はほぼ同等であり、また小児患者に対しては成人患者と比較して有効性が低いことも過去の報告と一致する結果であった。過去に MTX+VBL 治療の有効性と CTNNB1 変異型との関連を報告する研究はなく、本研究で初めて CTNNB1 変異型は MTX+VBL 治療の有効性に影響しないことが示唆された。しかし、MTX+VBL 治療を中止した 5 例中 1 例では腫瘍の再増大を認め、その腫瘍は S45F 型であったことは注目に値する。症例数を蓄積して解析を行うことにより、関連性の有無をより詳細に検討する必要がある。

E. 結論

日本人の腹腔外デスモイド腫瘍で他治療に抵抗性を示す症例に対して、2 週間間隔の低用量 MTX+VBL 治療は副作用が少なく、長期間継続可能な治療法である。S45F 変異型を有するデスモイド腫瘍に対しても有効性が期待できる治療である。

F. 研究発表

1. 論文発表

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デスモイド治療のアルゴリズム　メロキシカム抵抗性デスモイド腫瘍
第 87 回日本整形外科学会学術総会
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腫瘍径の大きなデスモイド腫瘍に対する治療アルゴリズム
第 47 回日本整形外科学会骨・軟部腫瘍学術集会 2014.7.17-18　大阪

G. 知的財産権の出願・登録状況
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厚生労働科学研究費補助金　難治性疾患等政策研究事業（難治性疾患政策研究事業）
分担研究報告書

腹腔外発生デスモイド腫瘍患者の実態把握および診療ガイドライン確立に向けた研究

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研究要旨　腹腔外発生デスモイド腫瘍は比較的まれな軟部腫瘍であり、手術後に高率に再発をきたし薬物治療の有効性も確立されていない。当科における腹腔外デスモイド腫瘍患者の30年間における診療実態を分析した結果、手術後の再発率は約50%と比較的高く、また四肢発生例では有意に再発率の上昇を認めた。また近年では初回治療として根治的手術が試みられる症例が過去に比し減少している傾向も明らかとなり、薬物治療の併用によって治療成績の向上が期待された。

A. 研究目的

腹腔外デスモイド型線維腫症は比較的まれな軟部腫瘍であり手術のみが根治手段であるが、他の良性腫瘍よりも高率に術後再発をきたすためWHO分類において中間群とされている。本邦におけるデスモイド腫瘍患者の診療ガイドラインは未確立であり、治療方針は各施設、各医師によって異なると考えられ、各施設における診療実態や治療成績を把握することは、将来的な診療ガイドライン作成において重要である。このため我々は、当科における過去約30年間における腹腔外発生デスモイド腫瘍患者の背景および治療成績を後方視的に分析した。

B. 研究方法

当科で1981年から2013年に治療を受けた腹腔外発生デスモイド腫瘍患者 33 名を対象とした。手術群と非手術群を比較し、手術群では再発の危険因子も検討した。全症例にて病理組織学的に診断を得た。手術群は21名、非手術群は12名であり、男性12名、女性21名であった。平均年齢は、手術群35歳、非手術群45歳であった。発生部位は四肢19例、体幹14例であった。手術群と保存的治療群を比較し、手術群では再発の危険因子も検討し、それぞれの群の治療成績を評価した。

（倫理面への配慮）

患者の受診様式、症状、治療方法、成績に関わる後ろ向き調査については個人情報の取り扱いに十分注意し、臨床研究に関する倫理指針（平成20年厚生労働省告示第415号）に準じて行った。

C. 研究結果

手術群と非手術群との比較において、発症年齢や性別、腫瘍発生部位に統計学的な有意差はなかった。平均経過観察期間は手術群82.7か月、保存的治療群27.0か月であり、有意に手術群で長期間であった。また手術群では平均腫瘍径が大きかった（腫瘍径 手術群 9.2 cm、保存的治療群 5.7 cm、$p = 0.0071$）。初回手術後の再発率は55.0%

であった。初回術式ごとの再発率は、広範切除群では47.1 %(n = 17)であり、腫瘍内切除例(n = 1)と辺縁切除例(n = 2)では100 %だった。初回手術後再発の危険因子を検討すると、四肢発生例における再発率は75%であり、体幹発生例における術後再発率25%と比較して有意に再発率が高かった。手術群全体でみた初回手術後の平均再発回数は1.4回であり、複数回再発例もみられた。手術群の転帰の内訳は、腫瘍なし生存15名、腫瘍あり生存5名、不明1名であった。非手術群の内訳は経過観察が6例、トラニラスト内服が3例、COX2阻害薬とトラニラスト内服が3例であった。転帰不明の2名を除いた治療成績は、経過観察例において腫瘍の増大なし3名および腫瘍の自然退縮が1名、COX2阻害薬使用例において腫瘍の増大なし2名および腫瘍の縮小が1名、COX2阻害薬とトラニラスト内服併用例において腫瘍の増大なしが2名および腫瘍の増大ありが1名であった。2004年以前の15名は全例手術が施行されており、2005年以降は手術群6名、非手術群12名と、近年では初回治療として手術が行われる症例が減少している傾向がみられた。

D. 考察

デスモイド腫瘍は高率に術後再発をきたすとされているが、本研究では初回手術後55%に再発を認めており、過去の報告と矛盾しない結果であった。術後再発の危険因子については若年発生、切除断端陽性、四肢発生例、再発腫瘍例などが報告されているが、本研究においては四肢発生例において有意に術後再発率の上昇がみられた。腫瘍なし生存、すなわち根治については手術例でのみ得られていたが、近年では単純経過観察例や初回治療として薬物治療が選択される例も増加傾向にあり、今後のデスモイド腫瘍治療の選択肢はますますの検討を要すると考えられた。

E. 結論

当科における過去約30年間の腹腔外発生デスモイド腫瘍患者33名に対して、発症様式、治療内容、治療成績を後方視的に検討した。根治は手術によってのみ得られるものの手術後の再発率は比較的高く、経過観察や初回治療として薬物治療が選択される症例が近年増加していた。四肢発生例においては術後再発率が特に高く、手術と薬物治療の併用などによって治療成績の向上が期待された。

F. 研究発表

1. 論文発表

なし

2. 学会発表

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G. 知的財産権の出願・登録状況

なし

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Hamada S, Nishida Y, et al.	Nuclear expression of β-catenin predicts the efficacy of meloxicam treatment for patients with sporadic desmoid tumors.	Tumour Biol.	35(5)	4561-6.	2014
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Ⅳ．研究成果の刊行物・別刷

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RESEARCH ARTICLE

Nuclear expression of β-catenin predicts the efficacy of meloxicam treatment for patients with sporadic desmoid tumors

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Abstract This study aimed to determine the prevalence of β-catenin nuclear positivity as a prognostic factor in patients with desmoid tumors (DTs) treated with meloxicam, a cyclooxygenase-2 (COX-2) selective inhibitor. Between 2003 and 2012, consecutive 31 patients with extraabdominal, sporadic DTs were prospectively treated with meloxicam as a systemic medical therapy. Immunohistochemistry was performed on formalin-fixed material to quantify the nuclear expression of β-catenin and Ki-67, and cytoplasmic expression of COX-2. All clinicopathological characteristics including the intensity of immunohistochemical staining were analyzed with respect to their prognostic value for meloxicam treatment. Of the 31 patients with meloxicam treatment, there was 1 with complete remission (CR), 7 with partial remission (PR), 12 with stable disease (SD), and 11 with progressive disease (PD). Higher nuclear expression of β-catenin was significantly associated with a poor response (PD/SD) (p= 0.017). The positivity of COX-2 and Ki-67 and none of the other clinical variables were associated with prognosis. The nuclear expression of β-catenin can predict the efficacy of meloxicam treatment for patients with sporadic DTs.

Keywords Desmoid tumor · Meloxicam · Prognostic factor · β-catenin · COX-2

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Introduction

Desmoid tumors (DTs), or aggressive fibromatosis, are mesenchymal tumors characterized by a local severely aggressive nature, but rarely metastasize, and do not cause disease-specific death if not located at anatomically critical sites [1, 2]. Extensive surgical resection has been the standard treatment for decades. However, radical surgical intervention and/or repeated surgery necessitated by a high recurrence rate (range 34–53 % at 5 years) [3, 4] may lead to significant treatment-related morbidity such as amputation or significant functional impairment. Several reports have failed to demonstrate any significance of the surgical margin on recurrence [3, 5, 6], while spontaneous regression has been reported in some patients [5, 6]. Together, the clinical course of DT is unusual and even enigmatic [7]. Several authors have recently outlined various conservative treatment modalities for DT including radiotherapy [8, 9] and drug treatment. Pharmacological treatment includes antihormonal agents, NSAIDs, and targeted and traditional cytotoxic chemotherapies [2, 10–13]. However, considering that tumor-related mortality is rare in patients with extraabdominal DT, cytotoxic agents with severe complications should be avoided as the initial treatment.

Cyclooxygenase-2 (COX-2) has been implicated as a factor in tumor initiation in colonic neoplasia and has also been demonstrated to play a role in the growth of DTs [14]. We have previously reported the clinical results of consecutive patients prospectively treated with meloxicam, a COX-2 selective inhibitor [12, 15]. The results of the studies showed that older age is a significant favorable prognostic factor for meloxicam treatment. However, there is difficulty in predicting the efficacy of meloxicam precisely before treatment. Biological markers are anticipated to be more useful in predicting the efficacy of this treatment.

β-catenin plays an important role in the development of desmoid fibromatoses and has the diagnostic potential to differentiate DT from other lesions [16]. The significance of β-catenin has been reported not only for the pathophysiological process of DT but also as a prognostic factor, particularly after surgical treatment [17, 18]. However, no useful prognostic factors have been identified for conservative treatment. Although we have reported favorable clinical results with meloxicam treatment for patients with extraperitoneal DT [12], the relationship between COX-2 and outcome has not been analyzed. This prompted us to investigate the prognostic value of β-catenin, Ki-67, and COX-2 for meloxicam treatment in patients with extraperitoneal DTs.

Materials and methods

Patients

We reviewed the medical records of 38 consecutive cases with extraabdominal or abdominal wall DTs diagnosed in our institutions since 2003. Seven cases were excluded. Two had been correctly diagnosed with other diseases. Two cases refused meloxicam treatment. Three cases had been followed for less than 6 months. There was no case with familial adenomatous polyposis-associated DT. Finally, this study was composed of 31 consecutive patients with extraperitoneal DT prospectively treated with meloxicam without any other previous treatment. No patients received other medical treatment or radiotherapy during meloxicam treatment. All 31 cases were histologically reevaluated as having DT. Meloxicam was administered orally at 10 mg/day. Baseline imaging of DTs by magnetic resonance imaging (MRI) and/or computed tomography (CT) was obtained before starting treatment. Patients treated with meloxicam have been followed with physical examinations and MRI and/or CT at the outpatient unit of our institution every 3–6 months. The efficacy of meloxicam was evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST) [19] measured with MRI or CT at the latest follow-up. When patients were evaluated as showing a complete response (CR), they discontinued meloxicam. Patients with partial response (PR) or stable disease (SD) continued meloxicam.

In cases with progressive disease (PD), patients can choose surgical treatment or low-dose chemotherapy with methotrexate and vinblastine, or continuation of meloxicam treatment after careful discussion of their tumor status. All patients signed an informed consent form, and the protocol was approved by the institutional review board of our institution.

Immunohistochemistry

All tumor samples were obtained at biopsy with no effects of meloxicam or chemotherapy. Biopsy specimens were fixed in 10 % formalin and embedded in paraffin. Paraffin specimens were cut at a thickness of 5 μm. The deparaffinized and rehydrated sections were treated with 0.3 % H_2O_2 in 30 % methanol for 30 min at room temperature to block the internal peroxidase activity, followed by incubation with 1 % bovine serum albumin in PBS as a blocking agent for 1 h at room temperature. The slides were incubated overnight at 4 °C with anti-β-catenin mouse monoclonal antibody (M3539; Dako, Carpinteria, CA; dilution, 1:200 dilution), anti-COX-2 goat monoclonal antibody (sc-1747; Santa Cruz Biotechnology, Santa Cruz, CA; 1:500 dilution), and Ki-67 mouse monoclonal antibody (M7240; Dako, Carpinteria, CA; 1:100 dilution). After rinsing with PBS, the sections were incubated with biotinylated secondary antibodies (Nichirei Biosciences, Tokyo, Japan), and the reaction products were observed using 3,3-diaminobenzidine tetrahydrochloride. Slides were counterstained with hematoxylin, dehydrated, and mounted [20]. Nonimmune mouse and goat serum was substituted for the primary antibody as a negative control.

Assessment for staining positivity

For β-catenin and Ki-67, positivity of nuclear staining was evaluated, whereas positivity of cytoplasmic staining was investigated for COX-2. Staining positivity was evaluated by two independent observers (S.H., N.F.) without any knowledge of the clinicopathological information and divided into 4 groups: 0 % for positive stainable cell number (negative; 0), 1–9 % (weak; 1+), 10–50 % (moderate; 2+), and 51–100 % (strong; 3+) on 10 independent high-power fields. We defined the positivity rate of β-catenin according to a previous report [21]. Ki-67 was divided into two groups; 0–1 % (negative; 0) and more than 1 % (positive; +). Using these criteria, both observers agreed on the degree of positivity or negativity of each case. Patients were divided into two groups as responders (CR, PR) or nonresponders (SD, PD). Age, gender, site (abdominal wall or extraabdominal), tumor size, and positivity of β-catenin, COX-2, and Ki-67 were examined as possible prognostic factors for responsiveness to meloxicam.

Statistical evaluation

Data were analyzed using the Chi-square test with Yates' continuity correction for dichotomous variables to examine correlations between the clinical results for meloxicam and clinicopathological characteristics including β-catenin, COX-2, and Ki-67 expression and clinical response. Continuous variables of age and tumor size were compared between the two groups using unpaired Student's t test. $p<0.05$ was considered significant.

Results

Clinical features

The mean age was 42.1 years (median, 37.0 years; range, 12–75 years). Eleven were male, and 20 were female. The anatomic distribution of the tumors was the abdominal wall in seven patients, four each in the neck and back, three each in the shoulder, thigh, and forearm, two in the chest wall, and one each in the upper arm, calf, foot, groin, and retroperitoneum. The diameter of the tumor ranged from 20 to 220 mm (mean, 83.7 mm; median, 76.0 mm). No patients had received radiotherapy or other treatment for DTs in advance of meloxicam treatment. The mean follow-up was 41.2 months (10–105 months). The median period of medication was 30.3 months (range, 2–105 months). Of the 31 patients evaluated, there was 1 patient with CR, 7 with PR, 12 with SD, and 11 with PD. There were no significant differences in age (p=0.44) or tumor size (p=0.85) between the responders and nonresponders. The number of analyzed patients increased in this study compared with our previous study [12]. It might cause that "age" was no longer a significant prognostic factor. The periods of meloxicam medication were 49 months at the time of CR evaluation and 28.0 months (median, range; 19–36 months) at the time of PR evaluation. These suggest that relatively longer duration of medication might be required to obtain good response.

Immunohistochemical findings

In all 31 cases evaluated, positive nuclear staining for β-catenin was observed; moreover, there was no case with weak positive (1+) (Table 1). Fourteen (45 %) patients showed moderate positive (2+) and 17 (55 %) strong staining (3+) (Fig. 1). Only one patient (13 %) in the responder group showed strong (3+), whereas 16 patients (70 %) in the poor responder group showed strong staining (3+). Of interest, none of the responders showed more than 60 % nuclear positivity of β-catenin.

All 31 cases revealed positive COX-2 staining in cellular cytoplasm. Four (13 %) patients showed weakly positive (1+), 12 (39 %) showed moderate (2+), and 15 (48 %) showed strong staining (Table 2) (Fig. 1). There was a trend in staining positivity between β-catenin and COX-2 (p=0.058). Among 31 cases evaluated for Ki-67 staining, 17 cases showed positivity equal to or less than 1 % (Table 3) (Fig. 1). Positive Ki-67 staining was not correlated with β-catenin (p=0.815) or COX-2 (p=0.301). There was no association between positivity of β-catenin and demographic data including age.

Table 1 Nuclear β-catenin expression and clinical prognosis

Intensity	CR	PR	SD	PD	Total
Negative (0 %)	0	0	0	0	0
Weak (1–10 %)	0	0	0	0	0
Moderate (11–50 %)	1	6	5	2	14
Strong (51–100 %)	0	1	7	9	17

Factors correlated with efficacy of meloxicam

Gender, age (<50 vs ≥50 ys), site (abdominal wall vs extraabdominal), and tumor size (<80 vs ≥80 mm) were not correlated with the efficacy of meloxicam treatment. Positivity of COX-2 staining and Ki-67 was not significantly correlated with responsiveness to meloxicam treatment. However, there was a statistically significant correlation with the intensity of nuclear staining of β-catenin (p=0.017), while strong β-catenin expression independently predicted a poor response to meloxicam with a relative risk of 1.88 (95 % CI, 1.10–3.22) (Table 4).

Discussion

β-catenin has been reported to play an important role in the Wnt signaling pathway involved in tumorigenesis in some tumors [22]. The accumulation of β-catenin caused by CTNNB1 (β-catenin) or APC mutation subsequently activates T cell factor, which in turn causes transcription of target genes in fibroproliferative disease [23]. In DT, this Wnt-β-catenin pathway also plays crucial roles in tumor development, and the nuclear expression of β-catenin has increasingly been used in the differential diagnosis of spindle cell neoplasms due to the high positivity rate of DTs [1, 16–18, 24]. The results of these previous reports are consistent with ours, in which all cases showed moderate (2+) or higher positive nuclear β-catenin expression.

Several previous studies reported the significance of nuclear expression for β-catenin in the prognosis of patients with DTs treated surgically. Gebert et al. analyzed nuclear expression for β-catenin in 37 cases with extremity and trunk DTs and reported that the overexpression of β-catenin is associated with a decreased event-free survival based on 23 cases with available clinical records. However, 6 of the 23 cases received surgery with a wide surgical margin and 17 with a marginal margin. Further, nine cases received radiotherapy primarily, suggesting that therapeutic bias may have influenced the results of β-catenin [17]. In contrast, Lazar et al. reported that the intensity of nuclear β-catenin expression was inversely correlated with the incidence of desmoid recurrence in the analysis of 195 tumor tissues. This study did not describe the details of surgical treatment or adjuvant treatment. Interestingly, the β-catenin 45F mutation exhibited a less intense β-catenin staining as compared with 41A mutation [18]. The association of β-catenin mutation status with

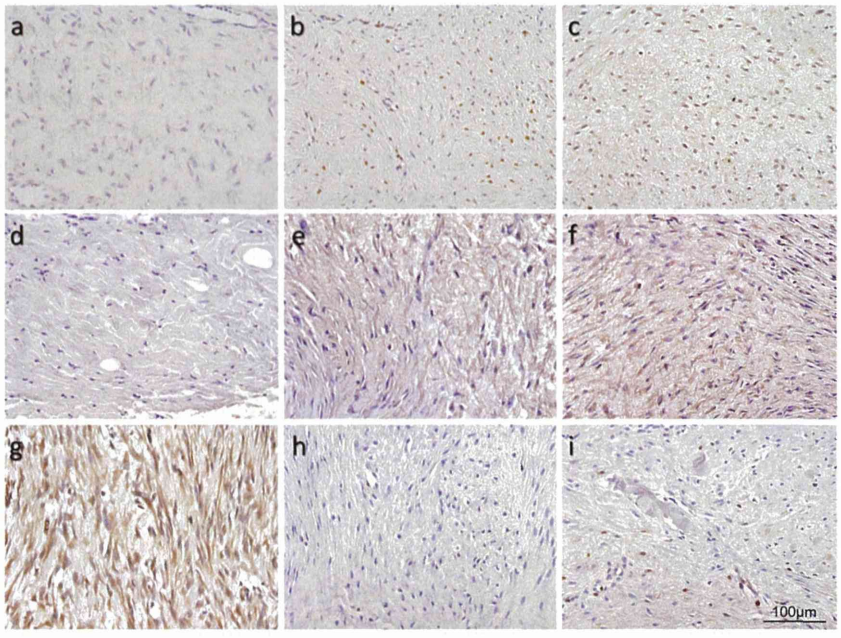

Fig. 1 Immunohistochemical staining of desmoid tumor. **a**, **b**, **c** β-catenin (**a** negative control; **b** moderate; **c** strong). **d**, **e**, **f**, **g** COX-2 (**d** negative control; **e** weak; **f** moderate; **g** strong). **h**, **i** Ki-67 (**h** negative; **i** positive) (counterstain with hematoxylin; original magnification, ×200)

positivity of nuclear β-catenin staining has not been clarified. On the other hand, Huang et al. showed that neither the percentage nor intensity of nuclear β-catenin expression correlated with tumor recurrence in their analysis of 46 cases [25]. In their study, only 6 of 46 cases (13 %) developed recurrence, representing a much better outcome than noted in other studies and suggesting that increasing the number of cases may produce different results. Together, these studies analyzing the significance of nuclear β-catenin staining for recurrence after surgery included heterogeneous and retrospective cohorts of patients. Prospectively designed studies may reveal more precisely the roles of β-catenin in surgically treated patients.

The treatment approach to sporadic DTs has recently changed. Bonvalot et al. proposed an algorithm that commences with more conservative modalities before resorting to treatments with predicted high morbidity [11]. We have prospectively treated sporadic DT patients with meloxicam since 2003. Although the early outcome was favorable [12], we have experienced an increasing number of patients who are resistant to meloxicam treatment. Predictive indicators for meloxicam treatment are required. There have been no informative reports analyzing prognostic factors for conservative treatment with not only COX-2 inhibitors but also hormonal agents. Bocale et al. reported that the response to antiestrogen therapy did not seem to be related to estrogen receptor status [26]. Therefore, we assessed the prognostic value of nuclear β-catenin expression in sporadic desmoid patients with meloxicam treatment. The present study demonstrated that patients with strong nuclear β-catenin staining are significantly resistant to meloxicam treatment, suggesting that this group of patients should be treated promptly with another modality. To the best of our knowledge, our results are the first to suggest that β-catenin might serve as a prognostic index for conservative treatment, particularly of COX-2 inhibitor. Moreover, the cohort of our study is composed of prospective and consecutive patients treated with meloxicam without any antecedent treatment, supporting the credibility of the results in this study.

COX-2 is an enzyme involved in prostaglandin synthesis. A previous study [14] showed that DTs express elevated levels of COX-2, which is consistent with the results in this study that the overexpression (more than weakly positive) of COX-2 was observed in all cases. Poon et al. demonstrated that COX-2 blocking decreased cell proliferation in desmoid cell cultures in vitro and the size of DTs in an in vivo mouse model [14]. Based on the preclinical study, following the IRB approval in our institution, we have applied the COX-2 inhibitor, meloxicam prospectively, to sporadic DTs as

Table 2 Cytoplasmic COX-2 expression and clinical prognosis

Intensity	CR	PR	SD	PD	Total
Negative (0 %)	0	0	0	0	0
Weak (1–10 %)	0	2	0	2	4
Moderate (11–50 %)	0	3	5	4	12
Strong (51–100 %)	1	2	7	5	15

Table 3 Nuclear Ki-67 expression (MIB-1) and clinical prognosis

Intensity	CR	PR	SD	PD	Total
Negative (0–1 %)	1	5	7	4	17
Positive (>1 %)	0	2	5	7	14

Table 4 Clinicopathological characteristics and clinical prognosis with responder (CR/PR) vs nonresponder (SD/PD)

Clinicopathological characteristics	Number	Responder (n=8)	Nonresponder (n=23)	p value
Gender				
Female	20	3 (15 %)	17 (85 %)	0.154
Male	11	5 (45 %)	6 (55 %)	
Age (mean=42.3 ys)				
<40 ys	18	4 (18 %)	14 (82 %)	0.464
≥40 ys	13	4 (36 %)	9 (64 %)	
Location				
Abdominal wall	7	1 (14 %)	6 (86 %)	0.764
Extraabdominal wall	24	7 (29 %)	17 (71 %)	
Size (mean=83.7 mm)				
<80 mm	17	6 (35 %)	11 (65 %)	0.359
≥80 mm	14	2 (14 %)	12 (86 %)	
Nuclear β-catenin staining				
Moderate (2+)	14	7 (50 %)	7 (50 %)	0.017
Strong (3+)	17	1 (6 %)	16 (94 %)	
Cytoplasm COX-2 staining				
Weak (1+)	4	2 (50 %)	2 (50 %)	0.475
Moderate (2+)	12	3 (25 %)	9 (75 %)	
Strong (3+)	15	3 (20 %)	12 (80 %)	
Ki-67 staining				
Negative (0)	17	6 (35 %)	11 (65 %)	0.359
Positive (+)	14	2 (14 %)	12 (86 %)	

conservative treatment since 2003. Nuclear accumulated β-catenin activates the T cell factor (TCF), which in turn causes transcription of target genes, one of which is considered to be COX-2 [1, 14]. However, the intensity of COX-2 expression was not correlated with the responsiveness to meloxicam but rather tended to be associated with nuclear β-catenin expression. There may be two possible explanations for this. One is that COX-2 comprises one part of the downstream of β-catenin/Wnt pathway which partially causes tumorigenesis of DT. This may be attributable partly to differences in the responsiveness to meloxicam treatment of each DT. Another explanation is the sensitivity of COX-2 immunostaining, since we did not confirm the COX-2 expression quantitatively with ELISA or immunoblot, which is a limitation of this study. Precise quantification of COX-2 may provide different information in the future.

In this study, the positivity of Ki-67 staining was very low and did not correlate with the positivity of β-catenin. This finding was consistent with that of a previous study and may reflect the essentially benign nature of DTs [27]. Brueckl et al. reported that low positivity of Ki-67 was of positive prognostic value concerning disease-free survival in a surgically treated series [28]. Considering that the increasing results of the relationship between nuclear β-catenin expression and treatment outcome, and very low positivity of Ki-67 staining in DTs, the prognostic significance of β-catenin might receive more attention.

There are some limitations in this study. First, the sample size was relatively small. However, given that DT is a rare condition and the cohort of this study is composed of prospective patients, the results derived from this study provide meaningful information. Future studies with more accumulated cases will help to clarify the association with the prognosis. Second, we evaluated only the expression of β-catenin and COX-2 immunohistochemically and did not perform quantitative evaluation of mRNA expression. Recently, other gene expressions were reported to correlate with the behavior of DT, including p53, EGFR, sex steroid receptor, and PDGF [18, 29–31]. Furthermore, the correlation of CTNNB1 gene mutation and prognosis has been reported in recent studies [17, 32]. Additional comparisons of such gene expression or mutation analysis with responsiveness to meloxicam may provide additional valuable information.

In conclusion, we suggest for the first time that nuclear β-catenin expression may serve as a prognostic marker in patients with sporadic DT treated with meloxicam. Given that treatment modalities for patients with DTs are now shifting to more conservative treatment, prognostificators for conservative treatment were urgently needed. Larger prospective studies are still necessary to confirm our findings and to further explore the role of β-catenin and other signaling in desmoid responsiveness to medical and surgical treatment.

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Conflicts of interest None

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CTNNB1 S45F Mutation Predicts Poor Efficacy of Meloxicam Treatment for Desmoid Tumors: A Pilot Study

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Abstract

We hypothesized that patterns of CTNNB1 (β-catenin) mutations would affect the outcome of conservative therapy in patients with desmoid tumors. This study aimed to determine the significance of CTNNB1 (β-catenin) mutations in predicting the treatment outcome in patients with desmoid tumors treated with meloxicam, a cyclooxygenase-2 (COX-2) selective inhibitor. Between 2003 and 2012, consecutive thirty-three patients with extra-peritoneal sporadic desmoid tumors were prospectively treated with meloxicam as the initial systemic medical therapy. The efficacy of meloxicam was evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST). DNA was isolated from frozen tissue or formalin-fixed materials. CTNNB1 mutation analysis was performed by direct sequencing. Positivity of nuclear β-catenin staining by immunohistochemistry was compared with the status of CTNNB1 mutations. The correlation between the efficacy of meloxicam treatment and status of CTNNB1 mutations was analyzed. Of the 33 patients with meloxicam treatment, one showed complete remission (CR), 7 partial remission (PR), 12 stable disease (SD), and 13 progressive disease (PD). The following 3 point mutations were identified in 21 of the 33 cases (64%): T41A (16 cases), S45F (4 cases) and S45P (one case). The nuclear expression of β-catenin correlated significantly with CTNNB1 mutation status ($p = 0.035$); all four cases with S45F mutation exhibited strong nuclear expression of β-catenin. S45F mutation was significantly associated with a poor response (all cases; PD) ($p = 0.017$), whereas the other mutations had no impact on efficacy. The CTNNB1 mutation status was of significant prognostic value for meloxicam treatment in patients with sporadic desmoid tumors.

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Introduction

Desmoid tumors, also known as aggressive fibromatosis, are mesenchymal tumors that show marked local aggressiveness, but rarely metastasize, and do not cause disease-specific death if they are not located at anatomically critical sites [1], [2]. Extra-peritoneal desmoid tumors, which are usually sporadic in nature, occur across a wide age range, and can arise at virtually any body site. Extensive surgical resection has been the standard treatment for decades. However, radical surgical intervention and/or repeated surgery due to a high recurrence rate (range 34–53% at 5 years) [3], [4] occasionally lead to significant treatment-related morbidity including amputation or significant functional impairment. Several reports have failed to demonstrate the significance of margin status in surgery for local recurrence [3–5], while spontaneous regression has also been reported [5], [6]. Several authors recently demonstrated the effectiveness of conservative treatment for desmoid tumors including radiotherapy and pharmacological treatment [7], [8]. Pharmacological treatment includes anti-hormone, NSAIDs, and targeted and traditional cytotoxic chemotherapies [2], [9–12]. However, the efficacy of these treatments cannot be predicted, and so remains a crucial problem.

β-catenin plays various important roles in the tumorigenesis of desmoid tumors, and has a diagnostic potential to differentiate them from other lesions [13], [14]. The nuclear accumulation of β-catenin causes activation of Wnt signaling, and in turn transcription of target genes in fibroproliferative disease [15]. Most desmoid tumors arise sporadically, with a minority associated with familial adenomatous polyposis (FAP), which is caused by a germline mutation of the adenomatous polyposis (APC) gene [15]. APC protein forms β-catenin destruction complex and is involved in the regulation of Wnt signaling. Several recent studies have reported point mutations of CTNNB1 (β-catenin) exon3. These mutations, occurring at codon 41 and 45, were found in about 64–85% of all sporadic desmoids, with p.T41A (threonine to alanine), p.S45F (serine to phenylalanine), and p.S45P (serine to proline) being the most frequent ones [16–19]. These mutations were considered to lead to stabilization of β-catenin and tumorigenesis in desmoid tumors, suggesting that the status of CTNNB1 mutations might influence the efficacy of various treatments for patients with these tumors.

We previously reported the clinical results of consecutive patients prospectively treated with meloxicam, a cyclooxygenase-2 selective inhibitor [12], [20], [21]. The efficacy of meloxicam treatment varied among patients, indicating that biological