特集 厚生労働科学研究成果報告書

【研究課題名】

がん医療ネットワークナビゲーターによるがん医療情報提供強化プロジェクト：情報が確実に手元に届く地域連携モデルの構築

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【研究区分】

厚生労働科学研究費補助金 疾病・障害対策研究分野 がん対策推進総合研究

【報告書区分】

総括

【研究年度】

平成 27（2015）年度

1. 研究目的

多くの患者にとって，がんとの闘いはすべてが未知の体験である．“知る”ことは医療と生活を選択するための基盤であるが，情報提供体制は十分ではない．診療と社会生活に関わる様々な情報を確実にすべての患者に伝える仕組みの確立は，がん患者が強く望む危急的課題である．その実現は，「がん対策推進基本計画」の全体目標である「全てのがん患者とその家族の苦痛の軽減と療養生活の質の維持向上」，「がんになっても安心して暮らせる社会の構築」，さらには分野別施策と個別目標となっている「地域の医療・介護サービス提供体制の構築」，「がんに関する相談支援と情報提供」に大きく貢献することになる．本研究では，がん診療連携機能の強化を大目的とし，地域がん医療ネットワークに精通した「がん医療ネットワークナビゲーター」の養成を試み，これを地域ネットワーク内に配置・機能させる情報提供の強化モデル事業を展開し，満足できるがん医療と社会生活を送るために適切な情報をすべての患者に確実に伝える仕組みの構築を目指す．

2. 研究方法

本研究は，がん医療ネットワークナビゲーターの，1）教育プログラムの確定とその遂行のための基盤整備，2）教育の実践と資格認定，及び3）資格認定者の現場配置によるモデル事業の実施と有用性評価，の3ステップからなる．平成26年度には，育成プログラムを確定し，教育ツール，研修，実習受け入れなどの準備を終了して募集を開始した．現在，座学とコミュニケーション研修を終了し，5月には実地研修を開始する予定である．平成27年度には，実際に資格認定を行う予定であったが，2016年6月には，第一期生の資格認定の見込みである．本モデル事業の評価を行うための評価委員会を立ち上げており，最終年度（平成28年度）には，実際に，がん年齢調整死亡率の低い（熊本），高い（福岡），中間（群馬）の3地域に「がん医療ネットワークナビゲーター」を配置して情報提供強化モデル事業を展開，効果，発展性，課題を検証して研究を総括する体

制を整備する.

3. 結果と考察

研究最終年度，平成28年6月には「がん医療ネットワークナビゲーター」が誕生する見込みである．最終年度（平成28年度）には，実際に，がん年齢調整死亡率の低い（熊本），高い（福岡），中間（群馬）の3地域に「がん医療ネットワークナビゲーター」を配置して情報提供強化モデル事業を展開する．地域ネットワーク内に配置・機能させる情報提供の強化モデル事業を展開し，満足できるがん医療と社会生活を送るために適切な情報をすべての患者に確実に伝える仕組みが構築できる．がんの医療とケアの面から厚生労働省の推進する医療介護，住まい，予防，生活支援サービスが身近な地域で包括される「地域包括ケアシステム」の確立に大きく寄与するとともに，がん患者の診療と社会生活に関わる様々な情報を確実にすべての患者に伝える仕組みの確立によって「がん対策推進基本計画」の推進，設定目標実現の促進に貢献するものと考えられる.

4. 結　論

本研究は，3年間で，がん診療連携機能と医療情報提供体制の強化をはかるために「がん医療ネットワークナビゲーター」を養成，その実効性を評価することを目指すものである．平成26年度は，1）e-ラーニングのコンテンツを確定して収録と監修を終了，2）教育研修セミナーを，群馬，福岡，熊本でセミナーを開催し，3）コミュニケーションスキル研修の要綱とともに，4）地域のがん診療・医療サービス，医療・生活支援サービスなどの情報を，過不足なく収集・提供するための実地研修の要綱とマニュアルを作成し，研修施設，指導者の認定作業を行った.「がん医療ネットワークナビゲーター」の養成基盤が確立でき，計画通り，平成27年4月から教育プログラムを稼働させることが可能となった．今年度は京都，熊本，福岡，群馬でコミュニケーションスキル習得のための研修を行い，さらに本研究において重要な役割を果たす研修施設の理解と協力をえる体制を整備し，実地研修の内容について検討を行った．その結果，群馬，福岡，熊本県を中心に40の研修施設の参加を得ることができた．最終年度早々に第一期の認定が可能な状況になっている．最終年度にはその実際の活動状況を報告するとともに，認定に関する体制およびその活動の評価を行う予定である.

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がん医療ネットワークナビゲーター

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臨　床　外　科
第71巻　第5号 別刷
2016年5月20日　発行

医学書院

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がん医療ネットワークナビゲーター

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はじめに

がん対策基本法のもと，推進基本計画が開始されて8年が経過する．計画は一定の成果をあげてきたものの，2007（平成19）年に掲げた10年間の全体目標である，がんの年齢調整死亡率（75歳未満）の20%減少は達成が難しいとされている[1]．また，患者の身体的，精神心理的苦痛の緩和がいまだ不十分であることや，医療の均てん化や医療情報の提供体制など，いまだ多くの課題が残されていることも明らかになっている．こうした課題に加えて，超高齢社会に突入した今，医療と介護，生活支援の連携強化も危急的課題となった．がん医療の場と，在宅医療と介護サービスを受ける患者の生活の場との間に大きな溝がある地域は決して少なくない．最初から最後まで満足できるがん医療を提供するには，従来にない包括的で効率的なアプローチが必要である．地域に密着した「がん医療ネットワークナビゲーター」養成事業は，そうした試みの一つである．

目的と役割

ナビゲーター養成は，がんの診療とケアの連携機能と情報提供体制の強化を目的としている（**図1**）．多くの患者にとって，がんとの闘いはすべてが未知の体験である．ここで強く求められるのは，切れ目のない医療とケア，そしてそれらを選択するために必要な様々な情報が適切に提供されることである．それらの願いはあまりに真っ当であるが，いまだ十分には叶えられていない．

医療連携を促進するために地域連携クリティカルパスの利用が推奨されてきた．しかしながら，今なお効果的に機能しているとは言いがたい．これを適応した患者数（延べ数）は明らかに伸び悩んでおり[2]，地域連携クリティカルパスを作成した際に加算されるがん治療連携計画策定料も，同パスを利用した際に加算できるがん治療連携指導料も，その請求額に著しい増加は認められない[3]．高齢化が進むなか，在宅医療・介護体制をいち早く整え，住み慣れた地域で拠点病院などとの連携を確保しつつ，療養生活を送ることができるような体制を確保する必要がある．効率的に機能する，在宅医療・介護体制とがん医療の緊密なネットワークの確立は危急的課題である．

がん医療に関わる情報も，確実に患者や家族の手元に届いているとは言いがたい．がん対策基本計画中間評価報告書では，“相談できる環境があると感じること”の割合は61.5%にとどまり，拠点病院のがん患者のうちがん相談支援センターを利用しているものの割合は，わずか7.7%に過ぎないことが示されている[1]．いずれも算出精度に課題の残る参考的な数字ではあるが，がん医療と介護を一貫するネットワークの構築も，がん情報の提供体制も，いまだ不十分と理解すべきであろう．

ここに，拠点病院相談員と協力し，がん拠点病院と

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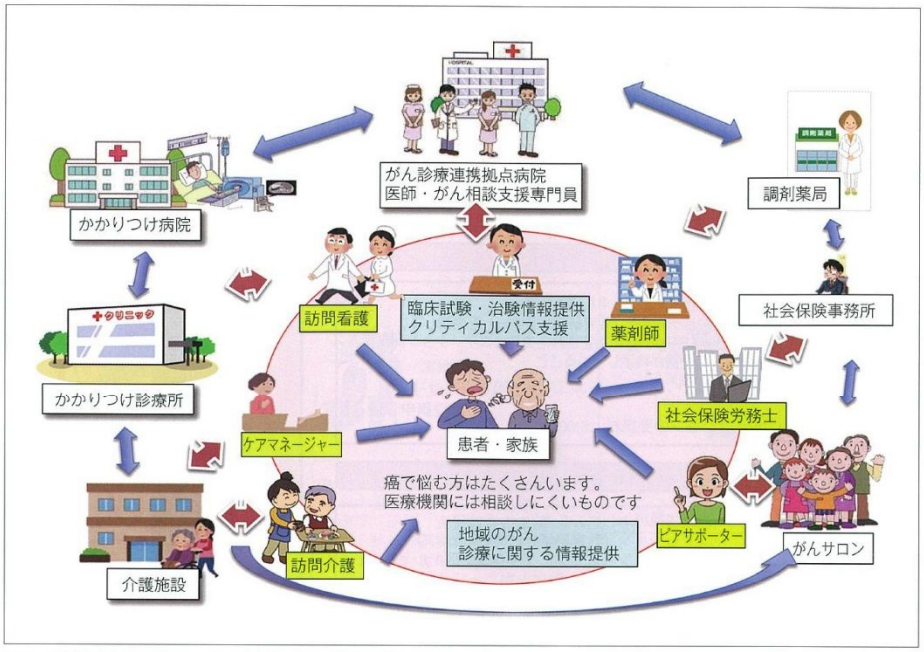

図1　がん医療ネットワークナビゲーター業務内容のイメージ

地域の在宅医療や介護サービスなどを確実につなぐ人材，がんに関わる医療・ケア・生活情報を確実に国民の手元に届ける人材，「がん医療ネットワークナビゲーター」を養成する試みが生まれた．いわば，患者や家族ががんに関わる様々な情報を効率的に得るための案内窓口である．その地域で，どこへ行けば適切な情報源へとたどり着け，有用な助言が得られるか，地域の情報に精通した案内人が身近にいることは極めて重要である．

がん診療連携拠点病院の相談支援センターを利用した者の満足度は81.4%にも達する[1]．このことは，相談支援センターへと確実に繋ぐシステムがあれば，患者・家族の不安を減らしうることを示唆する．しかしながら，患者・家族が求める情報は実に多岐にわたり，これらすべてに応えるには，医療機関，行政，介護など，おのおのの領域での専門的知識が必要である．拠点病院の相談員であっても，到底，一個人ですべてに対応できるわけではない．医療から介護まで一貫するためには，各領域の専門家たちへと適切に繋ぐ案内窓口が必要不可欠となる．

また，地域のがん患者を支えていくには，地域特性に応じて体制を構築する必要がある．地域密着型のがん医療ネットワークナビゲーターは，地域固有のがん医療ネットワークを熟知しており，その情報の収集や交換は，ネットワークの質の向上や拡大，さらには再構築をも促す可能性がある．さらには，検診やがん予防を促したり，地域におけるがん教育を支援したりする広報者や補助者としての役割も期待できる．

ナビゲーターの業務と育成

がん医療ネットワークナビゲーターの業務内容は以下の5点にまとめられる．①地域におけるがん診療情報や医療サービス情報を収集する．②がん患者・家族らの求めに応じ，がん診療情報や医療サービス情報を適切に提供する．③地域連携クリティカルパスの運用支援を行う．④臨床試験・治験に関する情報を適切に提供する．⑤医療介入またはこれに相当する可能性の

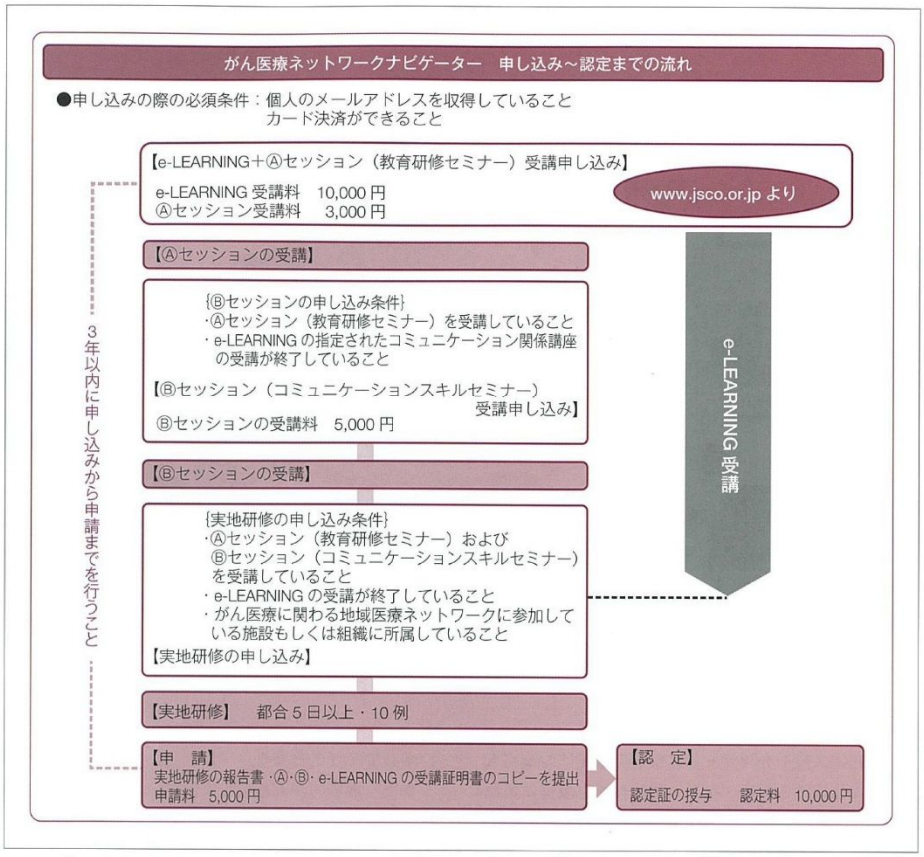

図 2　がん医療ネットワークナビゲーターの申請と認定　（日本癌治療学会 HP より）

ある行為は行わない．

これらの業務は，無償契約による守秘義務のもとに行われ，上記⑤にあるように特定の組織・機関の受診や治療を薦めたりするなどの医療介入は厳しく禁じられている．

ナビゲーターの認定を申請するには，一定の知識とコミュニケーションスキルの習得，実地研修が求められる．申請には，申請前 3 年間に，

(1) 日本癌治療学会の定める e-ラーニングシステムにおいて所定の科目を聴講し，すべての小テストに合格し，修了証を取得していること．

(2) 下記に定めるセミナー等（①，②，③）のうち，いずれか一つに参加し受講修了証を取得していること：①日本癌治療学会の開催するセミナー（A セッション：教育研修セミナー，B セッション：コミュニケーションスキルセミナー），②日本癌治療学会が認める研修　a）相談支援センター相談員基礎研修[1][2][3]，b）総合相談に携わる者に対する研修プログラムピアサポート編「これからピアサポートをはじめる人へ」の研修テキストを用いて開催される研修会のいずれか，ただし，a）を受講した者のうち，[1][2]まで履修済みで[3]が未修の者は①日本癌治療学会の開催するセミナー B セッションを受講しなければならない．b）を受講した者のうち，研修プログラムにロールプレイが含まれなかった場合は，①本法人の開催するセミナー B セッション

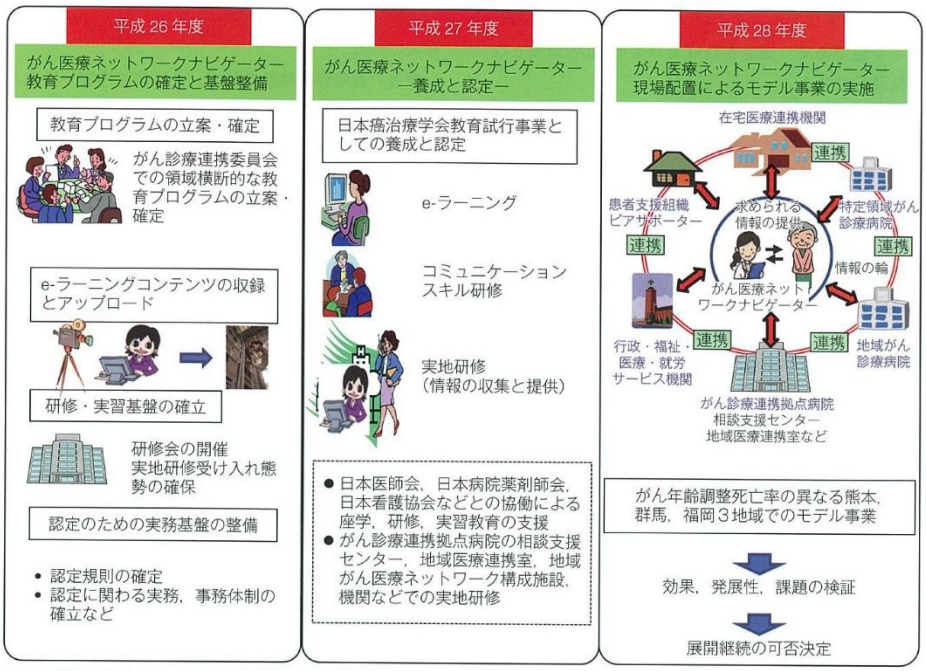

図３　がん医療ネットワークナビゲーター養成のモデル事業（計画）

を受講しなければならない．③このほか日本癌治療学会が認めるセミナー，研修会など．

(3) 日本癌治療学会の定める認定研修施設において，本法人の定める地域医療ネットワークの実地研修を修了し，指導責任者による証明がなされていること．

(4) 申請時にがん医療に関わる地域医療ネットワークに参加している施設もしくは組織に所属していること．が求められる．

（**図２**：http://www.jsco.or.jp/jpn/index/page/id/868）

職種は問わないが，地域の中で機能することが大前提であり，申請により拠点病院がネットワーク参加施設と認めた病院，医院，薬局，介護施設，ピアサポーター組織などのいずれかに属していることがその条件となる．

本制度は 2014 年度から 3 年間，厚生労働科学研究費補助金（がん対策推進総合研究事業）の支援を得て，日本癌治療学会のモデル事業として熊本，福岡，群馬の 3 県で開始され，その有効性の検証をもって次の段階，全国展開へと進む計画となっている（**図 3**）．現在，事業は順調に進んでおり，初年度（平成 26 年度）に教育プログラムを確立，2 年目となる平成 27 年度には，年度末の初回資格認定をめざして実働を開始した．

e-ラーニングの正式受講を開始し（平成 27 年 11 月 10 日現在，受講者 119 名），教育研修セミナー（A セッション）を京都で開催（平成 27 年 10 月 31 日，参加者 50 名：昨年度前倒開催と合わせ計 798 名），ロールプレイなどを含むコミュニケーションスキル研修（B セッション）では，ファシリテーター・マニュアル，テキストを作成して平成 27 年 11 月 1 日に京都大学で初回研修会を開催（参加者 22 名）した．同研修は，平成 28 年 2 月末までに，熊本，福岡，群馬と順次開催する予定になっている．

実地研修に関しては，要綱とマニュアルを完成し，福岡，熊本，群馬 3 県で説明会を実施して，計 36 施設を実地研修施設に認定するに至っている．平成 28 年 3 月より実際に各認定施設で研修を開始し，今年度末までに，所定要件をすべて修了した者を対象に，

「がん医療ネットワークナビゲーター」の初回認定に至る予定である．認定者数はおおよそ80名前後となる見通しである．

最終年度となる平成28年度には，「がん医療ネットワークナビゲーター」の養成を継続するとともに，資格取得者を，熊本，福岡，群馬の地域がんネットワークに実際に配置してモデル事業を展開する．その効果と発展性，課題を，実際の活動状況と利用者アンケート，拠点病院における相談件数などを用いて検証し，養成プログラムの修正を行うとともに，経済的背景についても検討して，今後の継続の可否について結論を導き，研究を総括する．

期待される効果

確実に国民の手元に届くがん医療情報の提供システムと，治療とケアが住まう地域で完結できる体制の確立は，「がんになっても安心して暮らせる社会」を実現するために必須の要素である．どのような病態であっても，また，入院中に限らず退院後も，求めることはいつでもどこでも知ることができれば，がんの医療とケアへの満足度は一気に向上する．

本モデル事業では，人材養成の質と継続性，および実践展開の円滑化を担保するため，研究代表者・分担者・協力者に日本癌治療学会の関連役員と実施担当者，研究支援者に日本医師会，日本看護協会，日本病院薬剤師会など，関係職能団体などの専門的役員を配している．

本事業の発展は，がん対策推進基本計画の全体目標である「すべてのがん患者とその家族の苦痛の軽減と療養生活の質の維持向上」の実現を大きく推し進め，分野別施策と個別目標である「地域の医療・介護サービス提供体制の構築」「がんに関する相談支援と情報提供」を可能とするものと思われ，また，職種を問わない人材養成により，経験を活かせる新たな雇用機会の創生に結びつく可能性もあり，この点でも期待をもっている．

今後，有効性の検証を経て，その業務内容，養成人数，資格取得条件などすべてにわたって再検討すべき，いわば胎児期の制度ではあるが，医療・保健上のみならず，社会的経済的にも大きく貢献するアプローチと考えている．

おわりに

人材育成は，学会などのアカデミアがなしうる直接的な社会貢献の一つであり，全うすべき重要な責務の一つである．超高齢社会への突入，がん多死社会の到来を目前に控える本邦において，本事業のもつ意味は極めて大きく，これを主宰する日本癌治療学会，なかでも，北川雄光理事長，本事業を統括するがん診療連携委員会の片渕秀隆委員長をはじめとする全委員，本研究事業の分担研究者である桑野博行，調　憲の両先生，研究協力者の相羽惠介，浅尾高行，境　健爾，佐々木治一郎，吉田　稔，藤　也寸志，竹山由子の諸先生の労をいとわぬ熱い志に心から敬意を表したい．

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FULL PAPER

BJC

British Journal of Cancer (2017), 1–9 | doi: 10.1038/bjc.2017.76

Keywords: gastric cancer; poor prognosis; chemosensitivity; Stathmin1

High STMN1 level is associated with chemo-resistance and poor prognosis in gastric cancer patients

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Background: Stathmin1 (STMN1) is a cytosolic phosphoprotein that regulates cellular microtubule dynamics and is known to have oncogenic activity. Despite several reports, its roles in gastric cancer (GC) remain unclear owing to a lack of analyses of highly metastatic cases. This study aimed to investigate STMN1 as a prognostic and predictive indicator of response to paclitaxel therapy in patients with GC, including inoperable cases.

Methods: Immunohistochemical analysis of STMN1 was performed on both operable ($n=95$) and inoperable GC ($n=61$) samples. The roles of STMN1 in cancer cell proliferation and sensitivity to a microtubule-targeting drug, paclitaxel, were confirmed by knockdown experiments using GC cell lines.

Results: Multivariate and Kaplan–Meier analyses demonstrated that high STMN1 was predictive of poor prognosis in both the groups. In the operable cohort, STMN1 expression correlated with cancer curability, recurrence, and resistance to adjuvant therapy. A correlation with paclitaxel resistance was observed in inoperable cases. Knockdown of STMN1 in GC cell lines inhibited proliferation and sensitised the cells to paclitaxel by enhancing apoptosis.

Conclusions: STMN1 is a possible biomarker for paclitaxel sensitivity and poor prognosis in GC and could be a novel therapeutic target in metastatic GC.

Gastric cancer (GC) is one of the most common malignancies globally, with ~989 600 (7.8% of the total) new cases and accounting for 738 000 (9.7% of the total) cancer-related deaths in 2008 (Jemal *et al*, 2011). Although the incidence of GC has been decreasing recently, its prognosis is generally poor with 5-year relative survival below 30% in most countries (Brenner *et al*, 2009). Surgery is the only curative treatment for patients with operable GC, and postoperative chemotherapy can improve the survival rate

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Received 1 January 2017; revised 26 February 2017; accepted 1 March 2017

after surgery (Cao *et al*, 2014). However, most patients are not eligible for radical surgery because of locally advanced or metastatic disease (Sugano, 2008). Therefore, it is important to identify predictors of poor prognosis and new therapeutic targets for patients with refractory GC.

Stathmin1 (STMN1), also known as oncoprotein 18, is a promising molecular target in several cancers and an important cytoplasmic phosphoprotein that regulates cellular microtubule dynamics. STMN1 promotes microtubule depolymerisation by sequestering tubulin (Marklund *et al*, 1996; Rubin and Atweh, 2004; Budhachandra *et al*, 2008) and stimulating catastrophes (Howell *et al*, 1999). High STMN1 expression is associated with poor prognosis in a variety of human cancers such as nasopharyngeal carcinoma (Cheng *et al*, 2008; Hsu *et al*, 2014), distal oesophageal adenocarcinoma (Akhtar *et al*, 2014a), oesophageal squamous cell carcinoma (Akhtar *et al*, 2014b), breast cancer (Golouh *et al*, 2008), hepatocellular carcinoma (Hsieh *et al*, 2010), cholangiocarcinoma (Watanabe *et al*, 2014), prostate cancer (Mistry and Atweh, 2006), colorectal cancer (Wu *et al*, 2014), and non-small cell lung cancer (NSCLC; Nie *et al*, 2015). STMN1 was suggested as a possible prognostic marker and a potential therapeutic target for GC (Jeon *et al*, 2010; Kang *et al*, 2012; Ke *et al*, 2013). In these previous studies, the immunohistochemical analyses of STMN1 expression were all performed on operable (resected) GC specimens and not on inoperable cases including locally advanced cases and those with distant metastasis.

With the development of molecular targeting agents, improvement in patient outcomes is expected in many cancers; however, significant progress has not been achieved in developing targeted therapies for advanced GC (Wong and Yau, 2012; Lee *et al*, 2014). Conventional cytotoxic agents are still the foundation of the treatment for advanced cases and paclitaxel, a microtubule-targeting drug, is one of the key therapeutics.

In this study, we performed immunohistochemical tests on human specimens to clarify the clinical significance of STMN1 in GC patients, including, importantly, biopsy specimens of inoperable tumours. We also conducted STMN1 suppression analysis to determine the effects of STMN1 expression on the proliferation, chemotherapeutic sensitivity, and paclitaxel-induced apoptosis of GC cells. Our results suggest that STMN1 expression could be used to predict the prognosis and therapeutic response to paclitaxel and would be a novel therapeutic target.

MATERIALS AND METHODS

Clinical samples and cell lines. We used 156 GC samples collected from 95 operable GC cases (resected tumour specimens from 77 men and 18 women) and 61 inoperable GC cases (endoscopic biopsy specimens from 42 men and 19 women; inoperable status determined at initial diagnosis). Of the 95 operable GC patients, 35 were treated with S-1 (Taiho Pharmaceutical Co. Ltd.; Tokyo, Japan) and 14 were treated with 5-FU-based chemotherapy after surgery. Of the 61 inoperable patients, 39 were treated with paclitaxel + S-1 and 22 were treated with cisplatin + S-1. S-1 (also known as TS-1) is one of the oral 5-FU-based anti-cancer drugs that combines tegafur, gimeracil, and potassium oxonate. The combination therapy of S-1 with cisplatin or paclitaxel is the standard regimen for inoperable GC patients in Japan (Mochiki *et al*, 2006; Satoh *et al*, 2011; Mochiki *et al*, 2012). All clinical GC samples were collected from Gunma University Hospital, Department of General Surgical Science between July 1999 to October 2011 and were used in accordance with the Helsinki Declaration and the guidelines of Gunma University Ethical Review Board for Medical Research Involving Human Subjects (approval number: 150044) after obtaining the written informed consent. The pathological features of the specimens were classified based on the 14th edition of the Japanese Classification of Gastric Carcinoma outlined by the Japanese Gastric Cancer Association. According to histology, the specimens were classified into differentiated type (well and moderately differentiated) and undifferentiated type (poorly differentiated and signet ring cells).

Human GC cell lines KATOIII, MKN7, MKN45 and MKN74 were maintained in RPMI 1640 containing 10% foetal bovine serum (FBS) and supplemented with 100 units per ml penicillin and streptomycin sulphate, and were cultured in a humidified 5% CO_2 incubator at 37 °C.

Immunohistochemistry. Paraffin-embedded blocks of the specimens were cut into 2 μm-thick sections and mounted on glass slides. All sections were incubated at 60 °C for 60 min and deparaffinised in xylene, rehydrated, and then incubated with fresh 0.3% hydrogen peroxide in 100% methanol for 30 min at room temperature to block endogenous peroxidase activity. After rehydration through a graded series of ethanol treatments, antigen retrieval was performed using Immunosaver (Nishin EM, Tokyo, Japan) at 98 100 °C for 30 min, and then the sections were passively cooled to room temperature. After rinsing in 0.1 M phosphate-buffered saline (PBS, pH 7.4), sections were incubated in Protein Block Serum-Free Reagent (DAKO, Carpinteria, CA, USA) for 30 min to block non-specific binding sites. The sections were incubated overnight at 4 °C with mouse monoclonal anti-STMN1 (OP18) antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a dilution of 1 : 100 in PBS containing 0.1% bovine serum albumin. The primary antibody was visualised using the Histofine Simple Stain MAX-PO (Multi) Kit (Nichirei, Tokyo, Japan) according to the manufacturer's instructions. chromogen 3,3-diaminobenzidine tetrahydrochloride was applied as a 0.02% solution in 50 m M ammonium acetate-citrate acid buffer (pH 6.0) containing 0.005% hydrogen peroxide. The sections were lightly counterstained with haematoxylin and mounted. Negative controls were incubated without the primary antibody, and no detectable staining was evident.

STMN1 immunostaining was evaluated independently by two experienced researchers and using the method described by Altan *et al* (2013). The method was based on the intensity and percentage of cytoplasmic or nuclear stained cells. The intensity was scored as follows: 0, no staining; 1+, weak staining; 2+, moderate staining; and 3+, strong staining (Supplementary Figure 1). The percentage of stained cells was calculated by examining at least 1000 cancer cells in five representative areas and was scored as follows: 0, no staining; 1+, 1–10%; 2+, 11–50%; 3+, 51–100%. The final grading was calculated by multiplying the intensity score with the percentage score. The lower grades (0, 1, 2, 3, and 4) were considered to be indicative of low expression, whereas the higher grades (6–9) were regarded indicating high expression.

Online microarray database search for STMN1 mRNA expression in GC. We used an online database KM plotter (www.kmplot.com) to validate the relevance of STMN1 mRNA expression to overall survival in patients with GC (Forster *et al*, 2011; Kim *et al*, 2012; Busuttil *et al*, 2014; Szasz *et al*, 2016). KM plotter is an entirely independent patient database, and a large scale survival data, which can be stratified by selected gene and characteristics including stage, Lauren classification, differentiation, gender, perforation, treatment, HER2 status, and data sets, can be available. We chose Affymetrix ID, 217714_x_at (STMN1), and investigated the prognostic value of STMN1 mRNA expression in 876 GC samples without above-mentioned characteristic restrictions. Auto select best cut-off value was used to identify the high and low groups. Overall survival data of 876 patients available were analysed by Kaplan–Meier survival curves (cut-off value was 361, and expression range of probe was 35–1473).

Protein extraction and western blotting. Total protein was extracted from KATOIII, MKN7, MKN45, and MKN74 cells using PROPREP protein extraction solution (iNtRON Biotechnology, Sungnam, Kyungki-Do, Korea). Total proteins were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) using Bis-Tris gels and were transferred to membranes by wet transfer. The membrane was blocked with 5% skim milk and incubated overnight at 4 °C with anti-STMN1 rabbit monoclonal antibody (1 : 1000, Cell Signaling Technology, Danvers, MA, USA) and β-actin mouse monoclonal antibody (1 : 2000, Sigma, St Louis, MO, USA). Bands on the membrane were detected with ECL Prime Western Blotting Detection Reagent using an Image Quant LAS4000 (GE Healthcare Life Sciences, Tokyo, Japan).

RNAi for down-regulation of STMN1. *STMN1*-specific siRNA oligos (STMN1 siRNA1; 5′-GAAACGAGAGCACGAGAAAtt-3′; STMN1 siRNA2; 5′-CGAGACUGAAGCUGACUAAtt-3′) and a non-targeting control siRNA oligos (NT siRNA) were purchased from Bonac Corporation (Fukuoka, Japan). MKN7 and MKN45 cell lines were seeded at 1×10^5 cells per well in a volume of 2 ml in 6-well flat-bottom plates and then incubated in a humidified atmosphere (37 °C and 5% CO_2) for 24 h. After incubation, 500 μl of Opti-MEM I Reduced Serum Medium (Invitrogen), 5 μl Lipofectamine RNAi MAX (Invitrogen) and STMN1 siRNA (50 nM final concentration in each well) were mixed and incubated for 20 min. The siRNA reagents were then added to the cells. The RNA interference assay was conducted after 24 h incubation.

RNA extraction and quantitative reverse transcription polymerase chain reaction (RT-qPCR). Total RNA was extracted from cells using the miRNeasy Kit (Qiagen, Hilden, Germany), and the quantity of isolated RNA was measured with an ND-1000 spectrophotometer (Nano Drop Technologies, Wilmington, DE, USA). RT-qPCR was performed using the GoTaq 1-Step RT-qPCR System (Promega, Madison, WI, USA) according to the manufacturer's instructions. The program consisted of four stages: reverse transcription at 37 °C for 15 min, reverse transcriptase inactivation and hot-start activation at 95 °C for 10 min, 40 cycles of 95 °C for 10 s, 60 °C for 30 s, and 72 °C for 30 s, and dissociation at 60–95 °C. The sequences of the primer pairs were as follows: STMN1 forward primer, 5′-AAGGATCTTTCCCTGGAGGA-3′; STMN1 reverse primer, 5′-CATTTGTGCCTCTCGGTTCT-3′; GAPDH forward primer, 5′-AAGGTGAAGGTCGGAGTCAAC-3′; GAPDH reverse primer, 5′-CTTGATTTTGGAGGGATCTCG-3′.

Cell proliferation assay. Proliferation analysis of MKN7 and MKN45 cells treated with NT siRNA or STMN1 siRNA was performed. The cells were seeded in 96-well plates (~3000 cells per well in 100 μl of medium containing 10% FBS). After 0 h, 24 h, 48 h, and 72 h, cell proliferation was measured with the Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Tokyo, Japan). Ten microliters of the cell counting solution was added to each well and incubated for 2 h at 37 °C. The absorbance of each well was determined using an Absorbance Spectrophotometer (Bio Rad, Hercules, CA, USA) at 450 nm with the reference wavelength set at 650 nm.

Paclitaxel sensitivity assay. Paclitaxel sensitivity of cells treated with NT siRNA or STMN1 siRNA was measured. The cells were plated in 96-well plates at ~8000 cells per well with 100 μl of medium, and after 24 h of incubation, the cells were treated with various concentrations of paclitaxel (0, 1.0, 10, 100, and 1000 nM) for 48 h. Cell viability was assessed using CCK-8 (10 μl per well, for

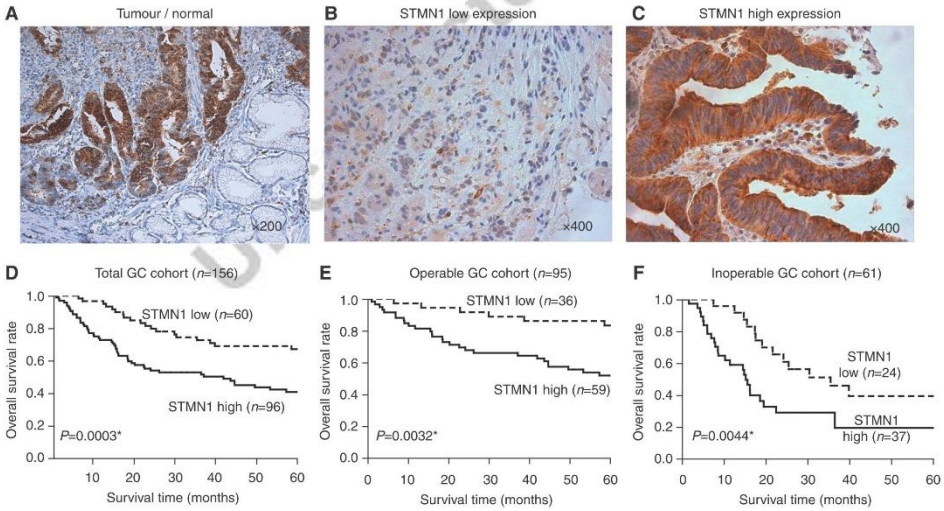

Figure 1. Immunohistochemical staining of STMN1 in GC samples. (**A**) Representative immunohistochemical staining of STMN1 in GC tissues (tumour) and normal gastric mucosa (normal; original magnification, × 200). The expression level of STMN1 was stronger in GC tissues than in normal gastric mucosa. (**B** and **C**) Low and high expression of STMN1 in GC specimens (original magnification, × 400). Sixty GC specimens (38.5%) were classified into the low-STMN1-expression group and 96 (61.5%) were assigned to the high-STMN1-expression group. (**D**) Kaplan–Meier overall survival in total GC cohort ($n = 156$); analyses were based on the expression of STMN1 ($P = 0.0003$). (**E**) Kaplan–Meier overall survival analyses of the operable GC cohort ($n = 95$) according to the expression level of STMN1 ($P = 0.0032$). (**F**) Kaplan–Meier overall survival analyses of the inoperable GC cohort ($n = 61$) according to the expression level of STMN1 ($P = 0.0044$). Kaplan–Meier overall survival rate in the high-STMN1-expression group was significantly lower than that in the low-STMN1-expression group.

2 h at 37 °C) and by measuring the absorbance of the medium at 450 nm with the reference wavelength set at 650 nm with an absorbance spectrophotometer (Bio Rad, Hercules, CA, USA). Paclitaxel was purchased from Sawai Pharmaceutical Co., Ltd.

Apoptosis assay. MKN7 and MKN45 cells treated with NT siRNA or STMN1 siRNA were seeded in 96-well plates. After 24 h, paclitaxel was added (paclitaxel concentrations: 0, 1.0, 10, and 100 nM) to the cells and incubated for 48 h. Paclitaxel-induced apoptosis was evaluated using the Amplite fluorometric Caspase-3/7 Assay Kit (AAT Bioquest) according to the manufacturer's instructions. Absorbance was read using the Enspire (Perkin Elmer) plate reader.

Statistical analysis. High-STMN1-expression group and low-expression group in clinical GC samples were compared using the Wilcoxon test, the χ^2 test, and the repeated-measures ANOVA. The Wilcoxon test was used to compare NT siRNA group with STMN1 siRNA group in *in vitro* analysis. Kaplan–Meier curves were generated for overall disease-free survival and statistical significance was determined using the log-rank test. Univariate and multivariate survival analyses were performed using Cox's proportional hazards model. A P-value of <0.05 was considered significant. All statistical analyses were performed using JMP software (SAS Institute Inc., Cary, NC, USA).

RESULTS

Immunohistochemical staining for STMN1 in GC specimens. We used immunohistochemistry to examine the expression of STMN1 in 156 GC specimens. The expression level of STMN1 was stronger in GC tissues (tumour) than in normal gastric mucosa (normal; Figure 1A). Among 156 GC cases, 60 (38.5%) GC specimens were classified into the low-STMN1-expression group

Table 1. Clinical factors and STMN1 expression from GC patients

Clinical factors	Total GC cohort ($n=156$)			Resected GC cohort ($n=95$)			Unresectable GC cohort ($n=61$)		
	Low $n=60$	High $n=96$	P-value	Low $n=36$	High $n=59$	P-value	Low $n=24$	High $n=37$	P-value
Age	64.4 ± 9.5	63.5 ± 10	0.7804	63 ± 9.4	65 ± 8.2	0.403	63.5 ± 10	65 ± 10	0.562
Gender									
Male	44	75	0.4956	28	49	0.524	16	26	0.7672
Female	16	21		8	10		8	11	
Histology type									
Well, moderate	33	45	0.3231	19	31	0.9822	14	14	0.1164
Poor, signet	27	51		17	28		10	23	
Tumour Depth									
m, sm	12	10	0.2388	12	10	0.1003	0	0	0.9086
mp, ss	20	33		17	28		3	5	
se, si	28	53		7	21		21	32	
Lymph node metastasis									
Absent	28	40	0.5404	18	27	0.6883	2	3	0.975
Present	32	56		18	32		22	34	
Liver metastasis									
Absent	56	90	0.9179	35	58	0.7253	21	32	0.9086
Present	4	6		1	1		3	5	
Peritoneal metastasis									
Absent	51	76	0.3568	35	55	0.3756	16	21	0.437
Present	9	20		1	4		8	16	
Clinical stage									
I	24	24	0.1218	24	24	0.0667	0	0	0.0314[a]
II	5	14		5	14		0	0	
III	13	17		6	14		7	3	
IV	18	41		1	7		17	34	
First treatment									
Sugery	36	59	0.856	—	—	—	—	—	—
chemotherapy	24	37		—	—	—	—	—	—
Surgical operation									
Absent	14	28	0.4214	—	—	—	14	28	0.1554
Present	46	68		—	—	—	10	9	
Curability									
Curative	46	63	0.1394	36	54	0.0264[a]	10	9	0.1554
Non—curative	14	33		0	5		14	28	
Recurrence									
Absent	—	—	—	28	26	0.0001[a]	—	—	—
Present	—	—	—	8	33		—	—	—
Clinical response									
PR	—	—	—	—	—	—	20	20	0.0395[a]
SD	—	—	—	—	—	—	2	12	
PD	—	—	—	—	—	—	2	5	

Abbreviations: PD = progressive disease; PR = partial response; SD = stable disease.
[a]Significant difference $P<0.05$.

(Figure 1B) and 96 (61.5%) were assigned to the high-STMN1-expression group (Figure 1C).

Clinicopathological significance of STMN1 expression of GC. Kaplan–Meier analysis of data from 156 GC patients demonstrated that the overall survival rate in the high-STMN1-expression group was significantly lower than that in the low-STMN1-expression group (Figure 1D). This was found to be the case in both operable ($P=0.0032$, $n=95$) and inoperable ($P=0.0044$, $n=61$) cohorts, classified according to the initial diagnosis of the 156 patients (Figure 1E and F). To confirm the prognostic significance of STMN1 expression in a large scale cohort, we used the KM plotter (www.kmplot.com), which includes published microarray data from 876 GC samples (Forster *et al*, 2011; Kim *et al*, 2012; Busuttil *et al*, 2014; Szasz *et al*, 2016). We validated that high expression of STMN1 in GC samples from a large database was associated with poor prognosis, the same as was found in our GC cohort (HR = 1.47, 95% CI = 1.22–1.77, $P<0.05$, Supplementary Figure 2).

Unexpectedly, clinicopathological analyses of STMN1 expression in GC revealed no significant correlation among any of the investigated factors in the overall GC cohort (Table 1). Nevertheless, high expression of STMN1 in operable GC patients was found to be significantly associated with poor cancer curability ($P=0.0264$) and recurrence ($P=0.0001$), whereas in inoperable cases, this parameter was shown to relate to the progression of clinical stage ($P=0.0314$) and poor clinical response against first-line chemotherapy ($P=0.0395$; Table 1).

Uni- and multi-variate regression analyses for overall survival, using data from 156 GC samples, indicated that high expression of STMN1 was an independent factor for poor prognosis (univariate analysis: RR = 2.49, 95% CI = 1.52–4.25, $P=0.0002$; multivariate analysis: RR = 2.79, 95% CI = 1.65–4.91, $P<0.0001$) and was associated with

Table 2. Univariate and multivariate analyses of overall survival in 156 GC patients

	Univariate analysis			Multivariate analysis		
Clinicopathological variables	**RR**	**95% CI**	***P*-value**	**RR**	**95% CI**	***P*-value**
Age						
≤65 *vs* >65	1.03	0.65–1.62	0.8976	—	—	—
Gender						
Male *vs* female	1.19	0.69–1.95	0.5078	—	—	—
Histology type						
Well, mod *vs* poor	2.08	1.31–3.36	0.0017[a]	1.79	1.129–2.92	0.0137[a]
Tumour depth						
SS *vs* SE, SI	20.3	4.48–357.6	<0.0001[a]	10.3	2.14–186.9	0.0011[a]
Lymph node metastatic						
Absent *vs* present	1.97	1.22–3.25	0.0048[a]	1.55	0.92–2.65	0.0942
Peritoneal dissemination						
Absent *vs* present	3.32	1.94–5.51	<0.0001[a]	3.06	1.74–5.24	0.0002[a]
Distant metastasis						
Absent *vs* present	2.65	1.15–5.28	0.0232[a]	2.01	0.86–4.14	0.1022
STMN1 expression						
Low *vs* high	2.49	1.52–4.25	0.0002[a]	2.79	1.65–4.91	<0.0001[a]

Abbreviations: CI = confidence interval; RR = relative risk.
[a]Significant difference $P<0.05$.

Table 3. Relationship between STMN1 expression and clinical factors (recurrence, clinical response)

STMN1 expression and recurrence in operable GC treated by adjuvant therapy ($n=49$)						
	S-1-treated operable GC ($n=35$)			5-FU-based medicine treated operable GC ($n=14$)		
Recurrence	**Low ($n=10$)**	**High ($n=25$)**	***P*-value**	**Low ($n=5$)**	**High ($n=9$)**	***P*-value**
Absent	5	4	0.0440[a]	2	3	0.8037
Present	5	21		3	6	

STMN1 expression and clinical response in inoperable GC ($n=61$)						
	Paclitaxel + S-1-treated inoperable GC ($n=39$)			Cisplatin + S-1- treated inoperable GC ($n=22$)		
Clinical response	**Low ($n=13$)**	**High ($n=26$)**	***P*-value**	**Low ($n=11$)**	**High ($n=11$)**	***P*-value**
PR	12	13	0.0141[a]	8	7	0.4836
SD	1	9		1	3	
PD	0	4		2	1	

Abbreviations: PD = progressive disease; PR = partial response; SD = stable disease.
[a]Significant difference $P<0.05$.

several cancer staging determinants, specifically, the histological type, tumour depth, and peritoneal dissemination (Table 2).

STMN1 expression and chemotherapeutic response. STMN1 is known to regulate cellular microtubule dynamics and its expression was confirmed to correlate with prognosis of GC patients. Based on these findings, we focused on the functional relevance of STMN1 to GC cellular sensitivity to chemotherapy, especially to paclitaxel treatment.

Of the 95 operable GC patients, 35 were treated with S-1 and 14 were treated with 5-FU-based medicine as an adjuvant therapy after the radical surgery. High STMN1 expression was significantly associated with a high recurrence rate ($P=0.044$) and poor prognosis ($P=0.0214$) in patients treated with S-1 after surgery (Table 3 and Figure 2A). These relationships, however, were not observed in patients treated with 5-FU-based adjuvant therapy (Table 3 and Figure 2B).

In the inoperable GC cohort ($n=61$), 39 patients were treated with paclitaxel + S-1 and 22 were treated with cisplatin + S-1 as first-line chemotherapy. High STMN1 expression correlated with poor clinical response ($P=0.0141$, Table 3) and poor survival ($P=0.0082$, Figure 2C) in the paclitaxel + S-1-treated group ($n=39$), but not in the cisplatin + S-1-treated group (Table 3 and Figure 2D).

Functional analysis of STMN1 in GC cell lines. We evaluated STMN1 expression in KATOIII, MKN7, MKN45, and MKN74 cell lines by western blotting (Figure 3A). We selected MKN7 and MKN45, which showed higher expression of the protein, for knockdown experiments to analyse the functional significance of STMN1 in proliferation and sensitivity to paclitaxel. siRNA was used to silence STMN1 and repression of the protein was confirmed by western blotting and RT–PCR (Figure 3B). Cell proliferation in the STMN1 siRNA groups was significantly suppressed compared to that in the NT siRNA groups and was closely associated with a decrease in STMN1 expression ($P<0.05$, Figure 3C). Cell viability in the STMN1 siRNA group decreased significantly following paclitaxel treatment compared to the NT siRNA group ($P<0.05$, Figure 3D). Furthermore, paclitaxel-induced apoptosis in the STMN1 siRNA group was enhanced more than that of the NT siRNA group. Determination of caspase-3/7 activities revealed that STMN1 knockdown enhanced paclitaxel-induced apoptosis. The number of apoptotic cells in the STMN1 siRNA groups after paclitaxel treatment was significantly higher than that in the other groups ($P<0.05$, Figure 3E).

DISCUSSION

In this study, we showed that high STMN1 expression was associated with poor prognosis in 156 GC patients including both cohorts of 95 operable and 61 inoperable cases. Thirty-nine and 22 inoperable GC patients were treated with paclitaxel + S-1 and cisplatin + S-1 combination therapies, respectively. We found that high STMN1 expression correlated to poor prognosis and poor response against chemotherapy in the paclitaxel + S-1 treatment group, but this correlation was not observed in the cisplatin + S-1

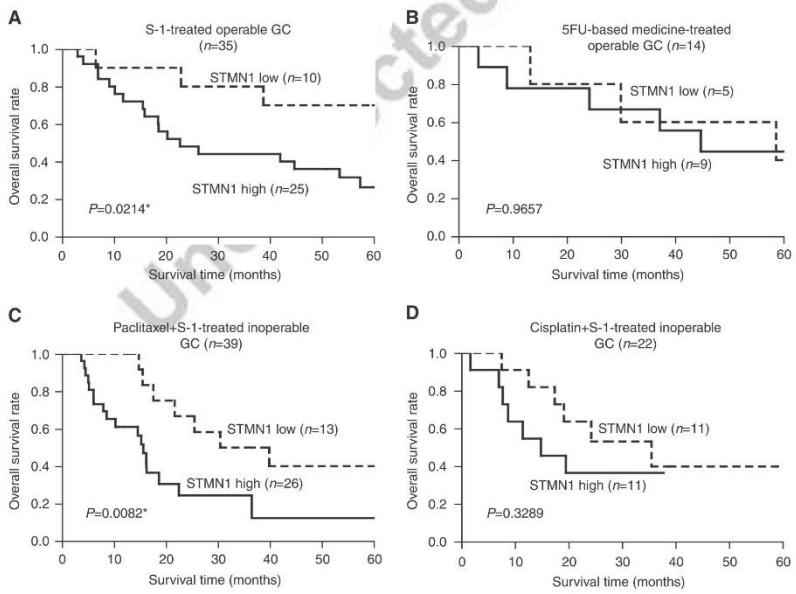

Figure 2. Overall survival curves of GC patients according to expression of STMN1. (**A** and **B**) Kaplan–Meier overall survival analyses of GC patients with operable tumours treated with S-1 and 5-FU-based medicine as adjuvant therapies after surgery. High STMN1 expression was significantly associated with poor prognosis in patients treated with S-1 after surgery ($P=0.0214$). However, this relationship was not observed in patients treated with 5-FU-based adjuvant therapy ($P=0.9657$). (**C** and **D**) Kaplan–Meier overall survival analyses in patients with inoperable tumours treated with paclitaxel + S-1 ($P=0.0082$) and cisplatin + S-1 ($P=0.3289$) as first-line chemotherapy. High STMN1 expression correlated with poor survival in the paclitaxel + S-1-treated group ($P=0.0082$), but not in the cisplatin + S-1-treated group ($P=0.3289$).

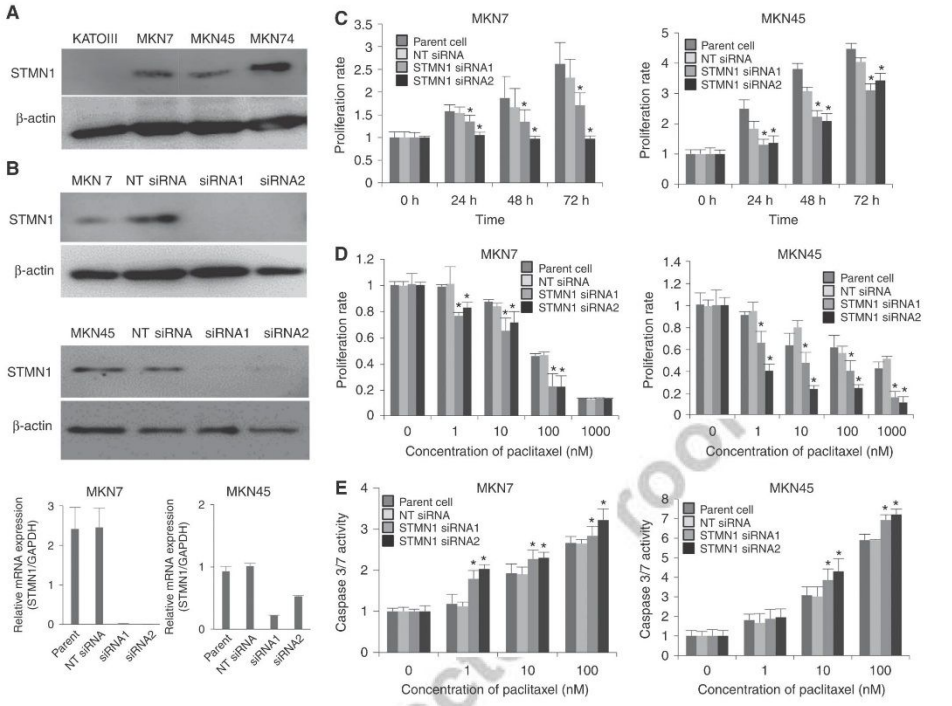

Figure 3. Functional analysis of human GC cell lines treated with STMN1 siRNA. (**A**) Expression of STMN1 was evaluated in GC cell lines KATOIII, MKN7, MKN45, and MKN74 by Western blotting. *β*-Actin was used as the loading control. (**B**) STMN1 expression in MKN7 and MKN45 cells treated with STMN1 siRNA1 or siRNA2 was detected by western blotting and RT-qPCR. STMN1 expression was suppressed in both STMN1 siRNA1 and siRNA2 groups. (**C**) Proliferation of MKN7 and MKN45 cells after STMN1 siRNA treatment was evaluated using Cell Counting Kit-8 kit. Cell proliferation in the STMN1 siRNA groups was significantly suppressed compared to that in the NT siRNA groups. (**D**) Paclitaxel sensitivity of MKN7 and MKN45 cells treated with STMN1 siRNA1 and siRNA2 was evaluated using Cell Counting Kit-8 kit. Cell viability in the STMN1 siRNA group decreased significantly following paclitaxel treatment compared to the NT siRNA group. (**E**) Paclitaxel-induced apoptosis in MKN7 and MKN45 cells treated with STMN1 siRNA1 and siRNA2 was evaluated by Amplite fluorometric Caspase-3/7 Assay Kit. Paclitaxel-induced apoptosis in the STMN1 siRNA group was enhanced more than that of the NT siRNA group.

treatment group. Moreover, multivariate analyses demonstrated that STMN1 expression was an independent prognostic factor in our cohorts. Our data suggests that STMN1 evaluation in GC tissues might be a useful marker for poor prognosis and chemosensitivity prediction.

In cancer patients, high STMN1 expression in tumours has already been reported to be associated with poor prognosis and more aggressive malignant potential than those with low STMN1 expression in tumours (Cheng *et al*, 2008; Golouh *et al*, 2008; Hsieh *et al*, 2010; Jeon *et al*, 2010; Kang *et al*, 2012; Ke *et al*, 2013; Hsu *et al*, 2014; Watanabe *et al*, 2014; Akhtar *et al*, 2014a; Akhtar *et al*, 2014b). These previous studies examined the significance of STMN1 expression only in resected cancer samples. On the other hand, our study evaluated the relationship between STMN expression, clinicopathological factors, and chemosensitivity in both resected GC samples and biopsy samples from inoperable GC patients. In this study, we clarified that high expression of STMN1 in the operable GC cohort was correlated with high recurrence rate after resection and advanced malignancy and high expression in the inoperable GC cohort correlated with advanced clinical stage and poor clinical response after chemotherapy. Our study is the first to demonstrate the possible clinical utility of STMN1 as a marker for both of operable and inoperable GC patients.

Wu *et al* (2014) reported that silencing STMN1 enhanced 5-FU sensitivity of colorectal cancer cells via a caspase-6-dependent mechanism. In addition, it was reported that STMN1 expression is related to the chemosensitivity to tamoxifen monotherapy in breast cancer (Golouh *et al*, 2008) and to platinum compounds and vinorelbine in NSCLC (Mlak *et al*, 2015). These observations suggest that STMN1 might be a drug sensitivity marker not only for taxane agents, but also for several conventional anti-cancer drugs. The association of high STMN1 expression with poor prognosis was observed in patients treated with S-1, but not in patients who received 5-FU-based adjuvant therapy. The limited number of patients in these treatment groups might have contributed to the low detection power of STMN1 compared to that in the other studies of cancer marker genes.

Previous studies have examined the association between STMN1 expression and the response to taxane therapy, and a

close association has been reported in ovarian cancer (Su *et al*, 2009), breast cancer (Alli *et al*, 2002), lung cancer (Yuan *et al*, 2012), and endometrial cancer (Werner *et al*, 2014). While studying its functional mechanisms, Iancu *et al* (2001) found that inhibition of STMN1 expression in erythroleukaemia cells increased the ratio of polymerised tubulin and the sensitivity to paclitaxel. Alli *et al* (2002) also reported that overexpression of STMN1 decreased polymerisation of microtubules and decreased sensitivity to paclitaxel by binding to paclitaxel and inhibiting the G2 to M transition of cells. Consistent with these reports, we found that STMN1 knockdown increased paclitaxel sensitivity and paclitaxel-induced apoptosis and that high STMN1 expression was associated with poor prognosis in inoperable GC patients receiving a paclitaxel + S-1 combination, but not in the cisplatin + S-1 group. Our data suggest that STMN1 expression is a predictive marker of the clinical response to combination chemotherapy treatment including taxane agents.

Candidates for targeted therapy against refractory cancers are believed to express cancer-specific profiles. In this study, we examined the STMN1 expression profiles in normal human tissues using an RNA sequencing database (RefEx [http://refex.dbcls.jp]). Expression was detected in only the testis and cerebrum, and not in other vital organs (Supplementary Figure 3). Consistently, we and other researchers have also found that the expression of STMN1 in cancer tissues is higher than that in normal tissues and that it is associated with poor prognosis and cancer progression in several types of cancers (Curmi *et al*, 1999; Rana *et al*, 2008; Nie *et al*, 2015; Saito *et al*, 2016). Moreover, knockdown of STMN1 in cancer cells decreased proliferation and increased taxane-induced apoptosis. A targeting strategy of cancer-specific STMN1 expression could be a promising universal therapeutic tool against refractory cancers including GC with STMN1 accumulation.

In summary, STMN1 expression is associated with cancer progression and chemo-resistance in clinical GC samples. STMN1 expression might be a prognostic marker for GC. STMN1 was also shown to regulate the proliferation and paclitaxel sensitivity of GC cells. Our results suggest that STMN1 expression in GC might be a useful prognostic marker and a promising candidate for targeted therapy.

ACKNOWLEDGEMENTS

We thank Ms Yukie Saito, Ms Tomoko Yano, and Ms Yuka Matsui for their excellent assistance.

CONFLICT OF INTEREST

Masahiko Nishiyama received a research grant from Yakult Honsha Co. Ltd. Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS): grant numbers 26461969, 15K10129, and 15K10085. The work was supported in part by Uehara Zaidan, and Gunma University Initiative for Advanced Research (GIAR).

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Supplementary Information accompanies this paper on British Journal of Cancer website (http://www.nature.com/bjc).