uncertain. Since relatives of patients with CRC are known to have higher risk of developing CRC, it is common practice to recommend they receive colonoscopy from a young age. However, we do not necessarily recommend that relatives of patients with gastric cancer undergo colonoscopy.

There can be a close association between the genetic predisposition to gastric cancer and CRC. The incidence of gastric cancer is very high in Japan, and the stomach is the most common cancer site in men and the second most common site in women [7]. Furthermore, gastric cancer and CRC can occur in the same individual. Indeed, gastric cancer is the second most common cancer occurring as the second primary cancer in patients with a history of CRC [9–11]. Gastric cancer is commonly encountered as Lynch-syndrome-related cancer, and even among those without Lynch syndrome, a family history of both gastric cancer and colon cancer is not uncommonly seen in Japan. Since it is considered that genetic predisposition is an important factor in the development of neoplasias in the young generation, we hypothesized that a family history of gastric cancer is associated with a high incidence of colorectal neoplasia in young Japanese.

To our knowledge, family history of gastric cancer has not been evaluated as a risk factor for the development of colorectal neoplasia. In this study, we conducted multivariate analysis to identify risk factors for the development of colorectal neoplasia in the young Japanese colonoscopy population, especially focusing on family history of gastric cancer. We also included other possible risk factors for colorectal neoplasia in the general population such as sex, body mass index (BMI) [12,13], cigarette smoking [12,14] and family history of CRC [5,6] in the multivariate analysis.

Methods

Patients

We conducted a retrospective multivariate analysis of young subjects under the age of 50 who underwent colonoscopy. Young subjects aged 30–49 years old (mean age 40.5) who underwent colonoscopy for the first time at Toyoshima Clinic during the period between August 2007 and August 2008 were included in this study. To minimize selection bias, 300 unselected consecutive patients who underwent colonoscopy for the first time were eligible, and we did not confine this to asymptomatic subjects but rather included both asymptomatic and symptomatic patients. Exclusion criteria were previous colonoscopy, inflammatory bowel disease (IBD) and family member with Lynch syndrome or familial adenomatous polyposis (FAP). The indications for colonoscopy were positive FOBT in 87 patients, haematochezia in 57 (three of whom were FOBT positive), other abdominal symptoms in 110 and medical check-up in 49 (Table 1).

Colonoscopy

Colonoscopy was performed using bowel preparation with polyethylene glycol or magnesium acetate. Sedation was usually obtained with midazolam or pethidine, or both, unless the patient refused it or a contraindication existed. If a neoplastic lesion was detected, polypectomy or biopsy was performed, and the diagnosis of adenoma or carcinoma was confirmed histopathologically. All procedures were performed without serious complications.

Data collection

The patients' charts and questionnaires were reviewed retrospectively, and family history of gastric cancer and CRC, age, sex, FOBT positivity, and presence of symptoms including haematochezia, abdominal pain, constipation and/or diarrhoea were evaluated. The positive family history was defined as having affected first- or second-degree relatives. Smoking history, non-steroidal anti-inflammatory drug (NSAID) intake, past medical history of diabetes and BMI were also evaluated.

Ethics

This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the ethics committee of the Institute of Medical Science, the University of Tokyo.

Table 1 Demographic and clinical features.

Feature	Number
Background	
Sex (male/female)	149/151
Age (30s/40s)	136/164
Positive smoking history	105
Positive family history of GC	56
Positive family history of CRC	45
BMI ($\geq$ 25 kg/m^2 *vs* < 25 kg/m^2)	51/249
Indication for colonoscopy	
Positive FOBT result	87
Haematochezia	57
Other abdominal symptoms	110
Medical check-up	49

GC, gastric cancer; CRC, colorectal cancer; BMI, body mass index; FOBT, faecal occult blood test.
Three of 87 with positive FOBT also noted haematochezia.

Statistics

Risk factors for the development of colorectal adenoma and/or carcinoma were assessed. Univariate and multivariate analyses were conducted using chi-squared and logistic regression tests with forward and backward stepwise selection, respectively. Factors with $P < 0.05$ were considered statistically significant. All analyses were performed using STATVIEW version 5.0 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Colonoscopy and detected lesions

No patient met the Amsterdam Criteria II. The caecal intubation rate was 100% in this series. A total of 118 colorectal neoplasias were detected in 83 (27.7%) subjects. Of these, two subjects were found to have invasive carcinoma and two mucosal carcinoma.

Univariate analysis

Univariate analysis revealed that older age, male sex, positive FOBT and a family history of gastric cancer were statistically significant risk factors for the development of colorectal neoplasia (Tables 2 and 3). Subjects with a first- or second-degree relative with gastric cancer had a significantly higher risk of development of colorectal neoplasia than those without a family history of gastric cancer (23/56 *vs* 60/244, OR 2.14, 95% CI 1.17–3.92, $P = 0.01$). Subjects above 40 years of age had a significantly higher probability of neoplasia than those younger than 40 (54/164 *vs* 29/136, OR 1.81, 95% CI 1.07–3.06, $P = 0.03$). Men had a significantly higher risk than women (49/149 *vs* 34/151, OR 1.69, 95% CI 1.01–2.82, $P = 0.046$). In terms of the indication for colonoscopy, FOBT status proved to be an independent risk factor. Since this study was analysed retrospectively, FOBT was not performed in those who underwent colonoscopy for other indications. Those with a positive FOBT result had a significantly higher risk than those with an unknown FOBT result (32/87 *vs* 51/213, OR 1.85, 95% CI 1.08–3.16, $P = 0.03$). BMI, smoking history and haematochezia did not reach significance. Since only six subjects took NSAIDs and three were diagnosed as having diabetes in this study, we could not assess them as risk factors.

Table 2 Detected neoplasia and adenocarcinoma against various risks.

Factor	Number	Neoplasia (%)	Adenocarcinoma (%)
Background			
Sex (male)	149	49 (32.9)	2 (1.3)
Age (40s)	164	54 (32.9)	3 (1.8)
Smoking history	105	34 (32.4)	2 (1.9)
Family history of GC	56	23 (41.1)	1 (1.8)
Family history of CRC	45	16 (35.6)	2 (4.4)
BMI (≥ 25 kg/m^2)	51	19 (37.3)	2 (3.9)
Indication for colonoscopy			
Any symptoms	167	39 (23.4)	1 (0.6)
Haematochezia	57	10 (17.5)	1 (1.8)
Positive FOBT result	87	32 (36.8)	2 (2.3)
Total	300	83 (27.7)	4 (1.3)

GC, gastric cancer; CRC, colorectal cancer; BMI, body mass index; FOBT, faecal occult blood test.

Table 3 Univariate analysis of risk factors for colorectal neoplasm.

Factor	Odds ratio	95% CI	*P*
Background			
Sex (male)	1.69	1.01–2.82	0.046
Age (40s)	1.81	1.07–3.06	0.03
Smoking history	1.43	0.85–2.40	0.18
Family history of GC	2.14	1.17–3.92	0.01
Family history of CRC	1.55	0.79–3.03	0.20
BMI (≥ 25 kg/m^2)	1.72	0.91–3.24	0.10
Indication for colonoscopy			
Any symptoms	0.62	0.37–1.03	0.06
Haematochezia	0.50	0.24–1.03	0.06
FOBT	1.85	1.08–3.16	0.03

GC, gastric cancer; CRC, colorectal cancer; BMI, body mass index; FOBT, faecal occult blood test.

Multivariate analysis

All of the significant risk factors shown in univariate analysis proved to be independent risk factors by multivariate analysis (Table 4). A family history of gastric

Table 4 Multivariate analysis of risk factors for colorectal neoplasm.

Factor	Odds ratio	95% CI	*P*
Age (40s)	2.05	1.18–3.55	0.01
Family history of GC	2.09	1.12–3.92	0.02
Family history of CRC	2.05	1.00–4.19	0.049
Sex (male)	1.89	1.10–3.27	0.02
FOBT	1.99	1.14–3.48	0.02

GC, gastric cancer; CRC, colorectal cancer; FOBT, faecal occult blood test.

cancer proved to be an independent risk factor (OR 2.09, 95% CI 1.12–3.92, $P = 0.02$), as well as male sex (OR 1.89, 95% CI 1.10–3.27, $P = 0.02$), older age (OR 2.05, 95% CI 1.18–3.55, $P = 0.01$) and positive FOBT (OR 2.00, 95% CI 1.14–3.48, $P = 0.02$). A family history of CRC appeared to be an independent risk factor in multivariate analysis, but the risk was not so strong (OR 2.05, 95% CI 1.00–4.19, $P = 0.049$).

Discussion

We conducted this study to clarify which individuals should receive colonoscopic surveillance for colorectal neoplasia before the age of 50. The most striking finding of the study is that family history of gastric cancer proved to be an independent risk factor for the development of colorectal neoplasias. According to the new American Gastroenterological Association guidelines [5], individuals with a first-degree family history of CRC or adenomatous polyps before the age of 60 are categorized as increased risk and should undergo colonoscopy at the age of 40. Those with two or more first-degree relatives with CRC or adenomatous polyps at any age are also categorized as increased risk. However, a family history of gastric cancer has never been considered as a risk factor for colorectal neoplasia. Our results indicate that individuals with a family history of gastric cancer in addition to CRC should undergo colonoscopy earlier than average risk individuals, since colonoscopy is now the gold standard screening tool for those with high risk for CRC and endoscopic resection of adenomas has proved to prevent a substantial proportion of CRC [15]. Although a family history of CRC was a less significant predictor than that of gastric cancer in our series, this may be partly due to the low power of the study or to the high incidence of gastric cancer in Japan.

Male sex proved to be an independent risk factor of the development of colorectal neoplasias in this study. Several studies have confirmed the same findings in older populations [16–18]. Brenner *et al.* accessed the US National Cancer Institute's Surveillance, Epidemiology and End Results programme and the World Health Organization mortality database and assessed national age- and sex-specific mortality data obtained from 11 large countries including Japan. They suggested gender-specific CRC screening recommendations. Our results support these findings in the young population.

Since rectal bleeding is one of the symptoms of colorectal neoplasia, individuals with haematochezia should undergo colonoscopy. In our series, haematochezia did not reach significance, while a positive FOBT proved to be an independent risk factor. Spinzi *et al.* [19] reported that only two of 312 patients aged 30–40 with haematochezia had malignant polyps. They concluded that patients younger than 40 rarely had advanced neoplastic lesions, and sigmoidoscopy appears to be sufficient. In contrast, Wong *et al.* [20] evaluated 223 patients under the age of 50 and found 12% of them had colon adenomas or cancer. They concluded that colonoscopy should be performed even in younger adults with haematochezia. In our series, our target lesions included not only carcinoma but also adenoma, so it is likely that those early lesions did not cause haematochezia. However, one of two patients with invasive carcinoma underwent colonoscopy because of haematochezia. Therefore, we agree with Wong *et al.* that those with haematochezia should undergo colonoscopy.

Faecal occult blood test is an established way to screen for CRC, and decreases both CRC and CRC-related death [21]. In our study, FOBT was performed elsewhere in most of the subjects. Furthermore, FOBT was not performed in subjects with other indications for colonoscopy. Therefore, we divided FOBT status into positive FOBT and unknown FOBT result. Those with positive FOBT proved to have a significantly higher risk of development of colorectal neoplasia than those with unknown FOBT result. Since FOBT cannot detect even advanced lesions, especially in the proximal colon, at times, we recommend from this study that individuals with a family history of gastric cancer should undergo colonoscopy earlier regardless of FOBT status.

Limitations exist in this study. Since this was a single-clinic-based retrospective study, selection bias cannot be excluded. To minimize selection bias, we chose 300 consecutive individuals who underwent first colonoscopy in the clinic over a 1-year period. Some previous studies included only asymptomatic populations to minimize bias [22]. Since we focused on the family history of gastric cancer and performed logistic regression analysis, we included not only asymptomatic individuals but also symptomatic or FOBT-positive individuals. Some of the CRC in the young were clearly associated with germ-line mutation, which is known in FAP and Lynch syndrome, and some were associated with IBD. Family members of patients with FAP or Lynch syndrome [5] and those with IBD [23] are recommended to receive colonoscopy from a young age, and it is not necessary to establish a screening strategy for these people. Therefore, those with high risk (e.g. FAP family, Lynch syndrome family, IBD) were not included in this study.

In conclusion, in the young Japanese population under 50 years of age, a family history of gastric cancer is an independent risk factor for the development of colorectal neoplasias. Large prospective studies are warranted to verify this finding.

Conflict of interests

The authors declare no conflict of interest. The study was approved by the ethics committee of the Institute of Medical Science, the University of Tokyo.

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Ⅸ 炎症性腸疾患の癌化

炎症性腸疾患に合併した小腸・大腸癌の特徴と外科治療

Clinicopathological features and surgery of intestinal cancer associated with inflammatory bowel disease

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Key words：潰瘍性大腸炎，クローン病，大腸癌，小腸癌，dysplasia

はじめに

本稿では，炎症性腸疾患患者においてサーベイランスや他の方法により癌やdysplasiaと診断がついてからの治療とそれに関する問題点を，日米欧のガイドライン[1-5]を中心として解説する．以下の文中で，ACGとはAmerican College of Gastroenterology，ECCOとはEuropean Crohn's and Colitis Organisation，BSGとはBritish Society of Gastroenterologyの略である．四角内に欧米のガイドラインとして示す文は監修された正式な和訳ではなく，便宜のため我々の日本語訳であることをお断りしておきたい．また，肛門(部)癌・痔瘻癌については他稿に詳しく記されているため，本稿では述べない．

1 潰瘍性大腸炎に合併した大腸癌の特徴と外科治療

a. 潰瘍性大腸炎に合併した大腸癌の特徴

1) 腫瘍か，それとも炎症性の'異型'か

【日本】潰瘍性大腸炎(UC)におけるdysplasiaに関する組織学的評価は，観察者間で異なることが少なくない：推奨グレードA(III b・8)

【ACG】腫瘍性のdysplasiaと炎症や修復による再生異型との明確な区別を確認するための病理学的な見直しを得ることが重要である．

【ECCO】病理医間での診断の相違率が高いためdysplasiaは経験のある病理医の確認をすべきである．

炎症が生じると上皮細胞は幼若化するため一見，腫瘍細胞と類似した像を示す．このためRiddellら[6]は炎症性腸疾患における腫瘍性病変の分類を示し対処法を提示した．更にこの論文では，病理医間における診断の違いも示している．すなわちこの論文に参加した病理医はいずれも経験を積んだ専門医たちであるが，その中でさえ同じ標本に対し異なった判断をしていることが示されている．著者らの知るかぎりその後の追試報告はなく，約30年前の結果をそのまま使用することの是非があるにせよ，この間に革新的な進歩はなかった．実地臨床では病理医が必ずしも消化管の専門でないことが多いことを考慮すると，少なくとも判断に迷う標本や，肉眼所見でdysplasiaが疑われる標本では経験を積んだ病理医の意見を求めるべきであろう．また著者らはdysplasiaが認められた場合にはできるだけ早期に再検査し，再度dysplasiaを確認する方がより確実と考えているが，ACG

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0047-1852/12/¥60/頁/JCOPY

ガイドライン[3]でも同様な記載がある.

2) 偶然の合併か大腸炎からの発癌か

炎症のため腫瘍が発生したとすると, その原因は局所的な問題ではなく同時性あるいは異時性の他の腫瘍発生が示唆される. 腸炎患者に偶然合併した腫瘍であれば, 次に発生してくる腫瘍の可能性が低いと予想される. 後で述べるように, どのように対処するかとの視点では両者の鑑別は極めて重要である. 鈴木らは年齢, 罹病期間, 罹患範囲, 腫瘍の形態などが有意に相違していたと報告した[7]. 例えば患者が若く罹病期間が長い症例に多発性で通常見ないような形態をした病変で癌が認められれば, まず間違いなく大腸炎から生じた癌であろうとの推定は可能である. しかし現実的には罹病期間が長い症例は同時にある程度年齢が高く, 通常の大腸癌があってもおかしくないような年齢であることも多いため, これのみではすべてを鑑別することは困難である. このため, 種々の方法が提案されてきた. 例えば味岡らはp53の免疫染色を施行し, dysplasia(腸炎を母地とした腫瘍)では75%の陽性率であるのに対し, adenoma(偶然の合併と考えられる腫瘍)では3.3%にとどまることから, 両者の鑑別に有用であるとした[8]. 今後の発展が期待される.

3) 腫瘍の多発性はどの程度認められるか

UCを母地とした腫瘍では同時性, 異時性の腫瘍が生じる頻度が高いことが知られている. 例えば, Cleveland Clinicでは同時性腫瘍は14%, 同時性dysplasiaは55%に認められた[9]. またMayo Clinicでは15%で同時性または異時性の複数の大腸癌が同定されている[10]. 正確を期すためには切除標本を全割しないかぎりその頻度は明らかにならないが, 実際には施行されていない場合が多いため本当の頻度はわからない[11]. 結局, 多重癌の危険は無視できるほど小さくないと考えるべきであろう.

4) Dysplasiaをどの程度診断できるか

dysplasiaは肉眼的な病変として認識できることも, 平坦粘膜に存在することもある[12]. Taylorらは95%のdysplasiaは平坦粘膜に認められるとしている[11]. 平坦な形態を呈したdysplasiaは隆起性形態を呈したdysplasiaと比べ発見しがたいことは言うまでもない. Bernsteinらも切除標本を完全に分析すると, 見逃されていたdysplasiaや癌が発見されることがある, としている[13]. 世界屈指の症例数と考えられるCleveland ClinicではUCを母地とした大腸癌の手術では5%に術前診断がつかなかった, と報告している[9]. どの数字が正しいかは別にして, 少なくともdysplasiaの診断が常に容易であるとは限らないことを考慮しなくてはならない. 実際, 著者らは明らかな隆起性病変を伴ったlow grade dysplasiaが炎症のためその存在すら不明となった症例を経験している. また色素内視鏡や拡大観察により内視鏡的な診断能力の向上は報告されているものの, 一般的な内視鏡医が施行したときの診断率はまだわかっていない. 残念ながら現状では内視鏡により確実にdysplasiaが検出できるという保証はないと考えられる.

5) 深達度診断の困難さ

UCに合併した大腸癌(以下, UC癌)ではしばしば細胞異型は軽度であるのに浸潤するような現象が認められる. 著者らは術前診断で明らかな浸潤癌と予想できなくても, 漿膜下層にまで浸潤した症例を経験している. また表層がUC-IIIであっても切除してみると浸潤癌であったこともあり, 標本の病理学的な所見では表層と浸潤先端部との悪性度の相違があった. このような場合にはいくら内視鏡で熱心に生検しても癌が検出されないわけで, 生検で癌が認められないことが癌でないとの証左にならないことはその取扱い上注意が必要である. Bernsteinら[13]も切除標本を完全に分析すると, 広がりや深達度を過小評価していたことに気づく, と述べている.

b. 潰瘍性大腸炎に合併した大腸癌の外科治療

1) 内視鏡的ポリペクトミー

実際の内視鏡的な手技を行うのは消化器内科医であることが多いと考えられるが, 物理的に腫瘍を取り除くことは'外科的'な手技にあたるため, ここで論じることにする. 腸炎のある領

域でポリープ状か腺腫様の病変が発見された長期経過UC患者に対する対処を述べた文献は多数ある[3]．

【ACG】もしその病変が内視鏡で完全切除され，周囲の平坦粘膜や他の部位にdysplasiaが認められなければ，長期経過を調べても癌のリスクは上昇しておらず，慎重なサーベイランス内視鏡による経過観察で十分である[14-19]．

【BSG】もしdysplasiaのポリープが炎症のあった部位で発見され完全摘除が可能であれば手術しなくともよい．この場合，周囲にdysplasiaがないことを確認すべきである．

コンセンサス会議では，腸炎を背景とした隆起性病変が腸炎を母地とした腫瘍か偶然の合併かとの判断に困ったときはポリペクトミーを行い，隣接した部位から4カ所の生検を加え，ポリペクトミーが完全に行われ周囲の生検でdysplasiaが認められず，他の腸管にdysplasiaがなければ6カ月以内に再検する[20,21]とされている．またACGのガイドラインでは，周囲の平坦粘膜や他の部位にdysplasiaが認められなければ偶然の合併として処理してもいいとの立場と考えられる．BSGのガイドラインでも周囲のdysplasiaの有無にその判断根拠を置いている．これは現実的にはUCを母地とした腫瘍か偶然の合併かを判断することが難しいため，便宜的に決められた基準と我々は理解している．

2）手術のタイミング

a）High grade dysplasia（HGD≒UC-IV）

【日本】大出血，穿孔，大腸癌合併が絶対適応となる：推奨グレードI（V・9）

【ACG】経験を積んだ病理医により確認されたHGDの診断は腸切除の適応である（evidence B）．

【ECCO】UCで平坦粘膜のHGDか癌が診断されたら手術適応である．［ECCO Statement 9J］

HGDは日本ではほとんどの場合‘癌’と診断される（厚生省研究班の基準でUC-IV）．日米欧いずれも，癌が認められれば手術適応だということで一致している．

b）Low grade dysplasia（LGD≒UC-III）

【日本】生検組織でdysplasiaを認め，更に他の消化管病理医により確認された場合には，通常大腸摘除術の適応となる：推奨グレードI（V・7）

【ACG】平坦粘膜のLGDも腫瘍の悪性化を予防するため手術適応になる可能性がある（evidence B）．

【ECCO】平坦粘膜のLGDは手術か3-6カ月後の再検査を行う．［ECCO statement 9J］

LGDの取り扱いについてはまだ決定的ではない．すなわち手術適応とする立場と通常より濃い密度のサーベイランス内視鏡を続けることを条件に保存的に経過をみる立場に分かれる．手術を考慮すべき理由はLGDが診断されてから5年以内にHGDや癌になる確率が54％と報告されているため[21-23]である．また，20のサーベイランスの成績をまとめた報告では，6カ月以内に腸切除をしたLGD患者の25％に癌があり，他の11％にHGDが認められた[24]．言い換えると，LGDはそれ自体がHGDや癌になるだけでなく，発見されていない他の部位の癌のマーカーとの側面もある．保存的経過をみる選択肢も示されているが，既に潜在的な癌があるかもしれないとの可能性を患者本人や家族に伝えておくことは必要であろう．

c）組織学的な異型が認められない場合

狭窄による症状があるか内視鏡が通過しない狭窄のある症例はしばしば長期経過例で大腸癌があることを示しているため腸切除が勧められる[3,25-27]．

3）術　式

通常の大腸癌と同様にUC癌でも部分切除で良かったとの報告はある．しかし，一般的には重症例や難治例と同様に大腸全摘が勧められている．実際，Cleveland Clinicでは大腸（亜）全摘72％，結腸全摘17％，部分切除9％，姑息的手術2％という術式が行われていた[9]．この主たる理由は既に述べたように，UC癌またはdysplasiaは同時性・異時性を問わず多発することが多く，その発見が確実とは言い切れないためである．このため癌・dysplasiaが手術適応

であるときの手術は，原則として大腸全摘＋回腸人工肛門造設術，大腸全摘＋回腸嚢肛門吻合術(IAA)，大腸亜全摘＋回腸嚢肛門管吻合術(IACA)のいずれかとなる．実際には高齢者や腫瘍残存切除例，腫瘍が肛門括約筋浸潤例を除いて，pouch operationが選択されることが多い．IAAとIACAのどちらを選択するかについてランダム化比較試験はない．しかしIACA後の癌合併症例報告では圧倒的にその前の手術適応が癌・dysplasiaであったことが多いため[28]，IACAは勧められず結果としてIAAを選択する方が無難と考えられる．しかし，IACAでもIAAと同等であるとの少数意見もある[29]．

4）予　後

無再発生存期間は同じステージの散発性大腸癌と同等とされてきた[9,10]．これに対し，近年のpopulation-basedや症例登録に基づいた検討では同じステージの散発性大腸癌と比べ予後が悪いと報告されている[30-33]．

2 クローン病に合併した大腸癌の特徴と外科治療

a. クローン病に合併した大腸癌の特徴

欧米ではクローン病(CD)に合併した大腸癌(以下，CD癌)は右側に多いとされている[34]．ClevelandClinicとMayo Clinicのデータではそれぞれ，直腸が35％，37％で右側結腸が30％，39％を占めている[9,10]．しかし我が国では61％とより多くの頻度で直腸・肛門部に集中している(詳細は他稿参照)．このように欧米と日本では明らかに癌の部位が異なっているためサーベイランスの有効性や癌に対する治療は欧米での基準をそのまま導入することが不適切となる可能性がある．

日米ではCDのガイドラインに癌・dysplasiaの対処に関する記載がないため，まず欧州のガイドラインを示す．

【ECCO】クローン病におけるdysplasiaや上皮性腫瘍の診断と悪性度の顕微鏡的な特徴は潰瘍性大腸炎に提案されたものと同様であり，確固たる診断のためにはセカンドオピニオンが勧められる(EL2，RG B)．[ECCO statement 3G]

【ECCO】潰瘍性大腸炎と同様に散発性の腺腫はDALMと鑑別困難なこともある．しかし散発性の腺腫と腸炎に伴ったdysplasiaの対処が異なるため，その鑑別は重要である．患者の年齢，腫瘍の部位と形態，周囲の平坦粘膜の生検所見が鑑別に有用であることがある(EL2，RG B)．[ECCO statement 3H]

このように欧州ではCDに合併する腫瘍性病変の特徴や取り扱いを，'UCと同様'という視点で済ませている．米国でもMayo Clinic[10]，Cleveland Clinic[9]，Cedars-Sinai病院[34]のいずれもUCの癌合併例とCDの癌合併例とに根本的な相違点を認めなかった．

UCでも問題となった腸管内の多重腫瘍に関しては，同時性腫瘍4-11％[9,10,35,36]，同時性のdysplasiaは30％[9]とまれではないが，UCよりは少ないかもしれない．

これに対し，我が国における報告例の集計[37]ではUC癌が大多数例で10年以上の罹病期間があるのに対し，CD癌では10年以下の症例も少なくないこと，癌の部位ではUC癌が通常の大腸癌とほぼ同じ分布を示しているのに，CD癌では上記のように直腸・肛門部により多く発生していることなどの相違点がある．

b. クローン病に合併した大腸癌の外科治療

欧米でもCD癌の治療に関するガイドラインの記載は少ない．

【ACG】外科治療はCD患者の2/3に必要で，難治性の出血・穿孔・持続性または再発性閉塞・膿瘍(経皮的な排膿不能)・dysplasiaや癌・重症で内科治療無効例が適応となる．

前項でも指摘したように，CDに合併した癌・dysplasiaはUCに準じて扱っていると考えてよいだろう．個別にみると，まず術前診断率は欧米で71％[9]となっていたのに対し，我が国では33/52(63％)であった．術式[9]は，欧米では大腸全摘が46％(UCでは72％)，結腸全摘が13％(UCでは17％)，部分切除が29％，姑息的手術が12％に行われており，有意にUCより大腸全摘が少なかった．これに対し，日本では結

腸全摘を含めても47例中4例にしか広範囲切除されていなかった[37]．欧米ではCD癌の予後は進行度を合わせた散発性大腸癌と同等といわれているが[3,9,10,34]，日本では詳細は不明ながら同じステージでも予後不良の傾向がある[37]．

3 クローン病に合併した小腸癌の特徴と外科治療

疫学的な検討からCDでは小腸癌のリスクが背景人口に比してオッズ比が15.64(95%CI: 4.26-40.06)[38]，17.4(95%CI: 4.16-72.9)[39]と非常に高くなっている．しかし著者らによる我が国の報告例の集計では18例と実数としては少ない[37]．癌の部位は記載のあった12例中11例で回腸となっており，一般的な小腸癌が回腸に多いことと，CDの炎症も回腸が中心であることを反映しているのであろう．CDとしての罹病期間は0-40(平均12)年と一般的には長期であり，UCに合併した大腸癌と同様に長期の炎症が小腸癌の発生にかかわっている可能性が考えられる．

診断時期は，記載されていた14例中，術前が2例にすぎず，術中に診断されたものが2例(14%)，術後の病理診断で初めて診断された症例が10例(71%)もあった．したがってCDの手術時には常に癌の可能性があることを念頭に置いて臨まなくてはならない．特に切除でなく狭窄形成を術式として選択したときには，ルーチンに病変部からの生検を行うことが勧められる．

上記報告例の中で小腸多重癌の報告は18例中1例のみである．この1例は3年後に直腸癌を合併し，他の1例でも異時性の大腸癌が診断された．組織型では記載のあった18例中，印環細胞癌が2例，低分化腺癌が3例と特殊な組織型が通常より多かった．深達度は12例中11例で漿膜下層かそれ以上と進行しており，肝転移や腹膜播種が同時に認められた症例も各1例含まれていた．また，dysplasiaについて検討してあった症例はごく少数であったが，4例中3例で同時にdysplasiaが認められた．

治療としては，記載例のすべてで切除が行われたが，化学療法が追加されていた症例も報告されている．

おわりに

炎症性腸疾患に合併した大腸・小腸癌について概説した．著者らが1995年にサーベイランスの問題点[40]を記してから患者人口が増え，長期経過例も著増したため，この分野への関心は大きく高まっている．内視鏡画像も著しく進歩し，分子生物学的な手法も随分一般化した．新たな展開を迎える日も近いと期待している．

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EXPERT OPINION

Targeting TLR3 with no RIG-I/MDA5 activation is effective in immunotherapy for cancer

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Introduction: Many forms of RNA duplexes with agonistic activity for pattern-recognition receptors have been reported, some of which are candidates for adjuvant immunotherapy for cancer. These RNA duplexes induce cytokines, interferons (IFNs) and cellular effectors mainly via two distinct pathways, TLR3/TICAM-1 and MDA5/MAVS.
Areas covered: We determined which pathway of innate immunity predominantly participates in evoking tumor immunity in response to RNA adjuvants.
Expert opinion: In knockout (KO) mouse studies, robust cytokine or IFN production is dependent on systemic activation of the MAVS pathway, whereas maturation of dendritic cells (DCs) to drive cellular effectors (i.e., NK and CTL) depends on the TICAM-1 pathway in DCs. MAVS activation often causes endotoxin-like cytokinemia, while the TICAM-1 activation does not. Unlike the TLR/MyD88 pathway, this TICAM-1 pathway barely accelerates tumor progression. Although the therapeutic effect in human patients of MAVS-activating or TICAM-1-activating RNA duplexes remains undetermined, the design of a TLR3 agonist with optimized toxicity and dose is an important goal for human immunotherapy. Here we summarize current knowledge on available RNA duplex formulations, and offer a possible approach to developing a promising RNA duplex for clinical tests.

Keywords: double-stranded RNA, MAVS (IPS-1, Cardif, VISA), immunotherapy, TICAM-1 (TRIF), TLR3

Expert Opin. Ther. Targets [Early Online]

1. Introduction

Toll-like receptor 3 (TLR3) was first identified in 2001 as a membrane-associated dsRNA sensor in TLR3-/- mice in which polyI:C-mediated NF-κB activation was severely hampered [1]. While it was suggested that signaling via TLR3, like TLR4, induced type I IFN [2], no conclusive data as to how this was achieved by dsRNA recognition were offered in that report [1]. We have established a mAb, TLR3.7, against human TLR3, that blocks dsRNA (polyI:C)-mediated type I IFN production in the human fibroblast line MRC5, and that demonstrates TLR3 localization on the cell surface membrane of these fibroblasts [3]. Moreover, immunoprecipitation analysis demonstrated that polyI:C-stimulated TLR3 formed a molecular complex with cytoplasmic proteins [3]. Ultimately, TICAM-1 (TRIF) was identified as the TLR3 adaptor [4]. By demonstrating that TLR3 recognizes dsRNA on the cell membrane and delivers an intracellular signal for the induction of type I IFN, these reports collectively link TLR3 to the type I IFN production pathways in fibroblasts in both human and mouse (**Figure 1A**).

RIG-I and MDA5 were soon discovered as cytoplasmic sensors for dsRNA that induced type I IFN [5,6]. It is reasonable that virus dsRNA replicating in cytoplasm is recognized by RIG-I/MDA5 of infected cells. What is the role of the

10.1517/14728222.2013.765407 © 2013 Informa UK, Ltd. ISSN 1472-8222, e-ISSN 1744-7631

Article highlights.

- dsRNA or polyI:C induces cytokines/IFNs through recognition by RNA sensors, TLR3 or RIG-I/MDA5.
- The cytoplasmic sensors RIG-I/MDA5 cause systemic cytokinemia in response to *in vivo* administration of polyI:C while the endosomal dsRNA sensor TLR3 does not.
- The TLR3 pathway resides in myeloid cells and mainly involved in DC-driven effector cells induction.
- TLR3-specific dsRNA stimulation with no involvement of RIG-I/MDA5 generates antitumor adjuvant activity without robust cytokine induction.
- TLR/MyD88 is a main pathway for NF-κB activation, which harbors protumor activity: yet, the TLR3 pathway does not involve MyD88 activation.
- Although polyI:C is too toxic to culminate driving cellular immunity against cancer, developing nontoxic antitumor adjuvants from derivatives of dsRNA would be feasible by redesigning dsRNA.

This box summarizes key points contained in the article.

TLR3 pathway for IFN induction in viral infection should have been reconsidered. PolyI:C has been historically used as an IFN inducer, and has been shown to engage both TLR3 and MDA5 [7,8]. Gene disruption studies in mice suggest that serum IFN levels in polyI:C-injected mice are increased primarily by the RIG-I/MDA5 pathway [7]. TLR3 is involved in local secretion of type I IFN limitedly in nearby tissues and organs (**Figure 1B**). Current understanding of this issue is that the fundamental function of TLR3 is not to induce robust IFNs/cytokines to alarm infection over whole body but to evoke cellular immunity, as mentioned below.

An alternative function of the TLR3 pathway has been clarified through studies of dsRNA (polyI:C)-stimulated or virus-infected tumor cells (**Figure 1A**). Cell death or growth is promoted in response to dsRNA, since TLR3 links to the RIP1 pathway to induce NF-κB activation and RIP1/RIP3-mediated cytolysis in tumor cells [9,10]. In addition, tumor-associated macrophages (Mf) (TAM) switches from a tumor-supporting to a tumoricidal phenotype in response to dsRNA [11]. Hence, the physiological role of the TLR3 pathway in tumor cells appears to provide dead cell-derived tumor Ag to DC and promote tumor immunity. Ultimately, these results suggest that if Ag and TLR3 agonist are provided for DC maturation, tumor cells expressing the Ag will be targeted by effector cells induced via the DC-derived immune response (**Figure 2**). Here, we focus on the role of dsRNA in evoking cellular immunity for cancer.

2. TLR3: distribution and localization

The gene encoding TLR3 is evolutionarily conserved across humans, mice, chickens and teleost fish [12-14] and TLR3 in all these species induces IFN production in response to polyI:C [13], implicating TLR3 as a potential viral dsRNA sensor in vertebrates [14]. However, viruses usually replicate in cytoplasm where no TLR3 is distributed. Furthermore, cell types expressing TLR3 are limited, at least in humans and mice, therefore TLR3 is unlikely to systemically protect tissues or organs from virus infection.

A human mAb against TLR3, TLR3.7, blocks dsRNA (polyI:C)-mediated type I IFN production in the human fibroblast lines where TLR3 localized on the cell surface membrane [4]. In contrast, the TLR3.7 mAb does not block the production of IFN by TLR3 in human monocyte-derived dendritic cells (MoDC) [15]. Electron or confocal microscopic analysis in these cells suggested that TLR3 is localized to endosomal compartment [15,16], which were later identified as early endosomes. Hence, human TLR3 is localized in a cell type-specific fashion, such that epithelial cells and fibroblasts, including MRC5 cells, express TLR3 on the cell surface, while in myeloid cells, TLR3 localizes to the endosome (**Figure 1A**). The surface-expressed type of TLR3 is positioned next to sample dsRNA in the extracellular milieu to transmit IFN-inducing signaling, whereas endosomal TLR3 engages phagocytosed dsRNA for signaling. Both types of endogenous TLR3 could be detected by TLR3.7 mAb by imaging analysis [15].

Since myeloid cells, including DC and Mf, take up external dsRNA into the endosome via phagocytosis [15-17], our current hypothesis is that endosomal TLR3 plays a role in sampling viral materials for augmenting antigen (Ag) presentation (**Figure 1B**). This model is gaining approval as the authentic adjuvant function of TLR3.

Human endosomal TLR3 is primarily expressed by myeloid cells, including dendritic cells (DCs) (**Table 1**) [18]. There are many subsets of DCs in humans, of which human CD141 $(BDCA3)^+$ DCs express high levels of TLR3 [19]. Human epithelial and endothelial cells express detectable amounts of TLR3 on their cell surfaces [3,20,21]. In contrast, tumor cells and malignantly transformed cells express TLR3 in endosomes rather than on their cell surfaces [18]. Tumor cells, therefore, unlike normal epithelial and endothelial cells, cannot directly sample environmental RNAs.

3. TLR3: structure and function

TLR3 is a type I membrane protein consisting of extracellular leucine-rich repeats and a cytoplasmic Toll-IL-1 receptor homology domain (TIR). The acidic environment in the endosomal lumen allows TLR3 to more tightly bind dsRNA than neutral cell-surface irrespective of the sequence. Forty to fifty base pairs of non-mismatched dsRNA interacts with homodimeric TLR3 almost exclusively with the sugar phosphate backbone rather than through the base moieties [22-24]. Intriguingly, the interaction of dsRNA with TLR3 does not trigger conformational changes, but rather facilitates homodimerization, which brings the intracellular TIRs of TLR3s in close proximity of each other [23]. TLR3 homodimerization recruits the adaptor TICAM-1 (TRIF) to the TIR domains [3],

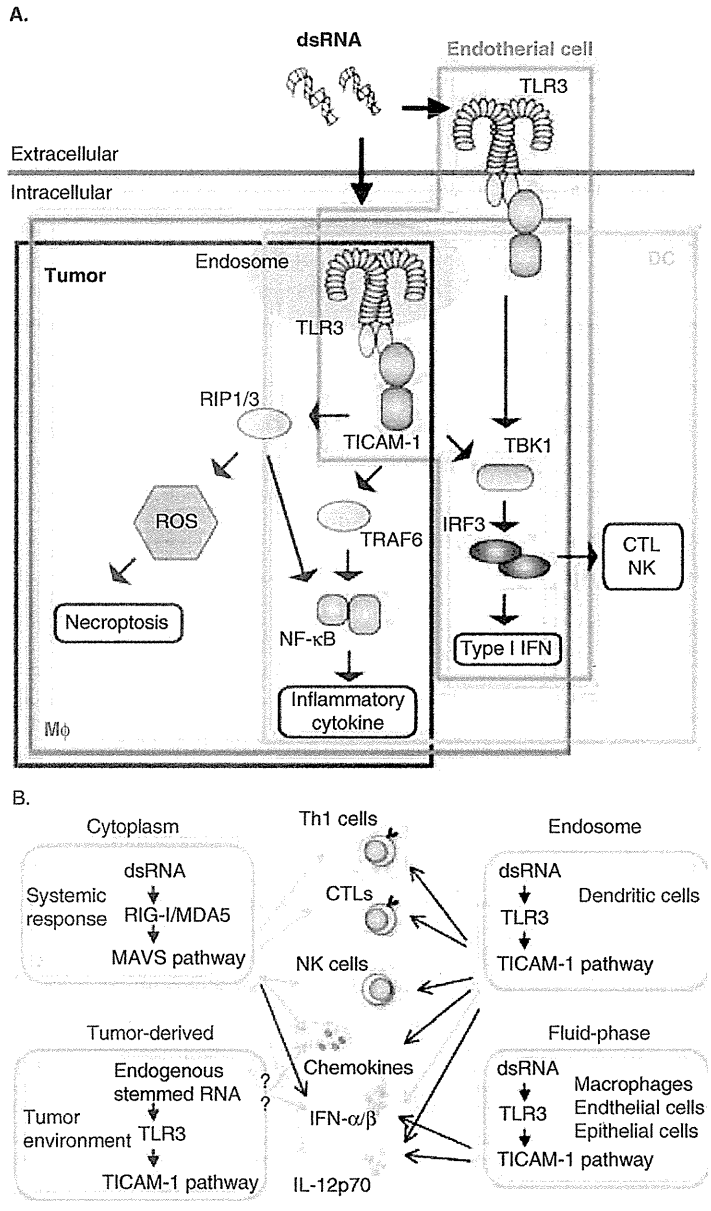

Figure 1. A. Cell type-specific activation of the TLR3/TICAM-1 pathway. Endothelial cells, epithelial cells and some macrophage subsets of human and mouse express TLR3 on the surface of the membrane and extracellularly sample naked double-stranded or stem/bulged RNA (red box). Tumor cells usually express TLR3 in the endosome and some cells activate the RIP1/3 pathway in response to dsRNA (black box). Most myeloid cells express TLR3 in the early endosome and take up debris-encapsulated dsRNA (blue and brown boxes). Cell death is induced in some tumor cells through the RIP1/3 pathway, which causes liberation of RNA-containing debris. Macrophages have unique properties of RIP1/3 and release DAMP. See the text for the functional properties of dendritic cell TLR3. **B.** A variety of output induced by dsRNA. Viruses produce dsRNA in the cytoplasm of infected cells during replication, and the cytoplasmic dsRNA is sensed by RNA sensors, RIG-I/MDA5 (left top panel). The cytoplasmic sensors contribute to production of robust type I IFN, leading to systemic cytokinemia, while they only weakly trigger other effectors without participation of the IFNAR pathway. On the other hand, dsRNA, either naked or encapsulated, can be incorporated into the endosomes of dendritic cells to induce cellular and soluble effectors (right top). Roles of surface-expressed TLR3 and endogenous stemmed RNA in this context still remain poorly characterized (bottom panels).

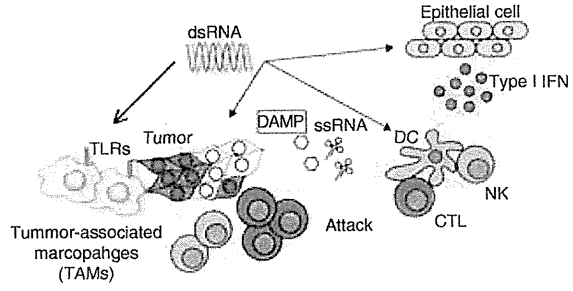

Figure 2. dsRNA-mediated inflammation modulates tumor microenvironment. A dsRNA or stemmed ssRNA affects tumor environment by acting on RNA sensors in epithelial cells, dendritic cells, tumor-infiltrating macrophages and tumor cells. Immune cells infiltrating the tumor mass may cause necroptosis of tumor cells. Tumor cells undergoing necrosis liberate DAMPs and debris containing Ag and nucleic acids with modified structural signatures of stem and bulge. These signatures can activate endosomal TLR3 in dendritic cells to promote the inflammatory response. Tumors in some cases benefit from the inflammatory response and in other cases regress in response to inflammation, and the mechanism determining this switch remains to be clarified.

which in turn recruits kinases TBK1 and IKK to the N-terminal domain of TICAM-1 [25]. The TICAM-1 complex thereafter dissociates from TLR3 and forms cytoplasmic speckles which are distinct from endosomes [26]. The kinase substrates IRF-3 and NF-κB are activated within the speckles, suggesting that the latter contain kinases and their substrates along with TICAM-1 [26,27]. After phosphorylation, IRF-3 migrates from the speckled region to the nucleus and acts as a transcription factor to induce type I IFN [27]. Without activation of the MAVS pathway, production of type I IFN is therefore a primary endpoint of dsRNA-mediated TLR3 activation in most cell types.

Notably, in some tumor cells and macrophages, ligand-stimulated TLR3 can facilitate RIP1 activation via the C-terminal TICAM-1 pathway, resulting in apoptosis or necroptosis (Figure 2) [28,29]. Furthermore, the TICAM-1 pathway triggers a chemokine/cytokine cascade via NF-κB activation that facilitates tumor progression in concert with the tumor microenvironment [30,31]. On the other hand, antigen-presenting cells (DC and Mf) express high amounts of TLR3, together with MHC and co-stimulatory molecules, and interact positively or negatively with lymphocytes. The primary function of myeloid cell TLR3 is to drive activation of NK cells by up-regulating surface-expressed NK-activating ligands (cell cell contact-mediated activation) [32] or induction of IL-12 and IL-15 (cytokine-mediated activation) [33]. Another function of myeloid cell TLR3 is to induce cross-presentation in DC and cross-prime antitumor CD8 T lymphocytes (CTL) [34]. Together, these functions of TLR3 are crucial for induction of antitumor cellular immunity (Figure 2).

4. Therapeutic TLR3 agonists

Three synthetic dsRNAs which harbor therapeutic potential as TLR3 agonists have been developed from polyriboinosinic polyribocytidylic acid (polyI:C), originally synthesized in the late 1960s to mimic viral responses [35]. IPH-3102, a dsRNA of unknown structure, appears to have a similar function to polyI:C [36]. In addition to these classical dsRNAs, single-stranded RNAs with nuclease-resistant stems are potential TLR3 agonists (Table 2) [37,38].

Ampligen (also known as rintatolimod) is a synthetic dsRNA consisting of polyI:C with one mismatch every 12 C, designating poly(I:C12U). It acts on DCs to induce tumoricidal effects, resulting in tumor growth retardation *in vivo* [39]. Ampligen, though has not been immunobiologically well-characterized as a specific agonist for TLR3, appears to operate in a multimodal fashion, encompassing the activation of natural killer (NK) cells, the proliferation of CTL, as well as direct cytostatic/cytotoxic effects on cancer cells. Hence, Ampligen targets a putative dsRNA sensor, most likely TLR3 across various cell types. Ampligen may not be a ligand for RIG-I/MDA5 [39].

Hiltonol (polyI:CLC) is a particular formulation of polyI:C that contains carboxymethylcellulose and poly-L-lysine as stabilizing agents [40]. Hiltonol is less vulnerable to degradation by serum nucleases or high temperature than Ampligen [41]. Hiltonol significantly elevates the levels of circulating IFNs in monkeys under conditions where an equivalent dose of Ampligen did not [40]. This has been attributed to the ability of Hiltonol to act as a ligand for both TLR3 and MDA5 [7,8] to exert potent immunostimulatory effects. Robust increases of serum type I IFN have been observed in multiple preclinical models, including mice and monkeys, although these are accompanied by an increased risk of side-effects [40-44]. Phase I/II clinical trials have been designed with patients having multifarious malignancies to assess the safety and efficacy of Hiltonol [45]. In general, these trials have concluded that low doses of Hiltonol are not particularly toxic in terms of cytokine induction and are moderately efficient in boosting antitumor immune responses. To date, ~ 20 Phase I/II clinical trials have suggested that Hiltonol is adaptable to immunotherapy for cancer in most cases, including brain tumor, malignant melanoma, breast cancer, and colorectal cancer [45].

Polyadenylic polyuridylic acid (polyA:U) is a synthetic dsRNA with immune-enhancing function *in vivo* on 1967 [46]. polyA:U was later found to stimulate TLR3 in Flt3-derived conventional DCs to generate IL-12 [47]. RIG-I/MDA5 recognizes only high amounts of polyA:U [46,48]. When combined with anticancer vaccines, poly(A:U) promotes Th1 responses that control tumor growth and are associated with the establishment of immunological memory [49]. On the other hand, poly(A:U) has protumor functions [50], because it stimulates TLR3 expressed on tumor cells to induce tumor cell proliferation [28,51-53]. There are currently no

Table 1. TLR repertoire in human dendritic cells.

Human TLRs	Monocyte	Freshly isolated mDC (BDCA1+)	Freshly isolated mDC (BDCA3+)	*In vitro*-differentiated cells pDC (BDCA4+)	*In vitro*-differentiated cells Monocyte-derived DCs
mAb					
TLR1 (1.136)	++	+	++	-	+
TLR2 (2.45)	++	++	+	-	++
TLR3 (3.7)	-	++	+++	-	++
TLR4	++	+	-	-	+
TLR6 (6.127)	++	+	++	-	+
TLR7		-	-	+	-
TLR8	+	+	+/-	-	+
TLR9	-	-	-	+	-

+: Protein or mRNA expression; Nucleotide-recognizing TLRs, TLR3, 7, 8 and 9 reside in intracellular compartments. MAVS pathway is ubiquitous while TICAM-1 pathway limitedly works in myeloid, epithelial and endothelial cells.

Table 2. Host response to RNAs and other DAMPs.

PAMP/DAMP receptors	
Microbial nucleic acids (PAMP)	
Cytosolic long dsRNA	MDA5
Cytosolic 5'-PPP-RNA	RIG-I
Endosomal > 140 bp dsRNA	TLR3
Fluid-phase dsRNA	TLR3
Encapsulated virus RNA	TLR3
Bulged or stemmed RNA	TLR3
Self nucleic acids (DAMP?)	
Modified self mRNA	TLR3
Some miRNA	TLR3
Denatured ssRNA	TLR3
Self molecular patterns (DAMP)	
HMGB1	RAGE, TLR2/4
Uric acid	CD14,TLR2/4
HSPs	CD14,TLR2/4
S100 proteins	RAGE

clinical trials evaluating its efficacy for either oncological or cancer-unrelated indications.

More exact information about clinical trials and oncological indications of these TLR3 agonists has been published by Galluzzi *et al.* [45].

5. Specific ligands for TLR3 without activation of the MAVS pathway

dsRNA and its synthetic analog, polyI:C, have long been known to be potent type I inducers [54] and modulators for cellular immunity [55]. Indeed, mouse and human versions of TLR3 recognize dsRNA and transduce TICAM-1 signals for NF-κB and IRF-3 activation. Type I IFN/cytokine and cellular immunity induced by the cytoplasmic dsRNA receptors RIG-I/MDA5 and NLRP3 have been identified more recently [45,48,56]. The type I IFN production induced by dsRNA is largely attributable to the MAVS pathway rather than the TICAM-1 pathway [7]. Furthermore, KO mice studies with *in vivo* administration of polyI:C have suggested that dsRNA contributes to NK cell activation and CTL proliferation even in MAVS-/- and IFNAR-/- mice, the initial response to which is independent of MAVS- or IFNAR-mediated type I IFN production [34,57]: i.e., tumor-specific NK cell and CTL can be induced without increasing serum type I IFN level in mice. Hence, the activation of cellular immunity occurs irrespective of the serum level of IFN in tumor-bearing host, although IRF-3 is essential for cellular immune activation [57].

Regarding the question as to through which target receptor, MDA5 or TLR3, polyI:C induces antitumor cellular immunity, evidence suggests that it is TLR3 [32,34]. Previous studies demonstrated that both MDA5 and TLR3 were equally associated with initiation of cellular immunity in response to i.p. injection of polyI:C + Alum in mice [58]. However, initial IRF-3 activation in myeloid cells is closely linked to NK cell activation but not robust IFN induction [57-59]. In syngenic mouse tumor-implant models, TLR3-TICAM-1 is more important than MDA5-MAVS for CD8α$^+$ DC to evoke antitumor cellular immunity below the protumorigenic polyI:C dose [32,34].

The optimal *in vivo* doses for induction by polyI:C of type I IFN, NK activation or CTL induction (cross-priming) are as yet unknown. It is likely that the optimal dose for inducing type I IFN, largely attributable to the RIG-I/MDA5 pathway, differs from those for facilitating NK activation and/or cross-priming induced by human DC [19], particularly a subset of a human counterpart of mouse CD8α$^+$ DC, namely CD141$^+$ DC. In both human and mouse DCs, 10 μg of polyI:C activates NK cells to kill tumor cells *in vitro* [60]. However, the dose discrepancy appears to cause different immune responses between human and mouse with respect to *in vivo* polyI:C administration. In the C57BL/6 mouse, 1 μg of i.p. injection of polyI:C per mouse is sufficient to induce type I IFN and IFN-inducible genes in spleen cells, but is insufficient

for causative NK/CTL activation to effect regression of implant tumors [32,34,61]. Likewise, in human volunteers, 1.6 mg of s.c. polyI:C has been shown to induce type I IFN in whole blood [62]. It is currently unknown whether this dose is sufficient for activation of NK/CTL in humans. The reported doses were restricted by the toxicity of polyI:CLC, and may have been sufficient for RIG-I/MDA5 activation followed by the feedback activation of the amplifiable IFNAR pathway in mice and humans, however, the dose has not been determined to optimize IRF-3-dependent NK/CTL activation by Ag-presenting DC.

NK cell activation requires > 10 μg of polyI:C per mouse, although the quality of polyI:C, including the average length of the duplex region, varies and critically affects the optimal dose for induction of cellular immunity [62,63]. For induction of cross-priming in mice, > 50 μg/20 g is actually required by i.v. or i.p injection. With regard to s.c. injection of dsRNA, several shots in different areas would be ideal for administration of the dsRNA reagent. If high-dose administration of dsRNA is also mandatory for induction of cellular immunity in humans, the dose 1.2 1.6 mg/volunteer would be a short dose in humans. If the dose limitation of polyI:C in human trials is mostly due to side-effects such as cytokinemia and protumor activity, the development of less toxic RNA reagents is indispensable for facilitating human immunotherapy.

There are several points of concern in the context of high-dose polyI:C therapy. Firstly, the likelihood of a cytokine storm is increased in healthy volunteers receiving > 1.6 mg polyI:C due to systemic activation of the MAVS pathway. Erythema, arthralgia and general malaise have been reported and may be secondary to elevated type I IFN [64]. The other point concerns the protumor activity of the TICAM-1-RIP1 pathway. Appropriate doses that neither activate the RIP1 pathway in tumor cells nor induce tumor growth should be chosen for antitumor therapy. Moreover, the duration of the effects is currently also unknown, although a single-shot dsRNA has only a short duration over ED_{50}. IFN-α/β levels may be kept high, being sustained by the IFNAR pathway [65]. TLR3 is endosomally expressed in myeloid DCs as well as in tumor cells. The types of cell involved in the immune response against high-dose polyI:C remain undetermined in humans, and the role of RNA-sensing receptors in other cell types therefore warrants further exploration.

6. Other RNA derivatives in tumor environment

Recent reports have suggested that single-stranded (ss)RNAs with incomplete stems serve as ligands for TLR3 [37,38]. ssRNA with a ~ 200 bp duplex may act as a TLR3 agonist without activation of MDA5 [24,63]. As mentioned above, the capacity of ssRNA to activate TICAM-1, but not MAVS, makes it suitable for antitumor immunotherapy, since it has only marginal cell-proliferative activity but fully activates NK cells and CTL in relevant tissues with induction of only low levels of IFN. The results are promising in the context of the synthesis of TLR3-specific ligands which do not participate in the MAVS pathway, and which can be applicable to humans without marked toxicity.

It has been believed that viral dsRNA is liberated from virus-infected cells through cell death events, apoptosis or necrosis. Oncogenic viruses may trigger death signals by activating cytoplasmic RNA sensors in transformed cells. An EB virus RNA with an incomplete stem, named EBER, also activates TLR3 [66] and, together with RIG-I, induces live signals and sometimes accelerates tumorigenesis in infected hosts [67]. Alternatively, transformed cells release live signals in the form of type I IFN and proinflammatory cytokines (IL-6, IL-12, TNF-α, etc.), which are liberated through IRF-3/7 and NF-κB activation as the output from living virus-infected cells. TLR, NLR and other cytosolic nucleic acids sensors are closely associated with RNA recognition (**Table 2**), and inflammation states are therefore fundamentally variable and individually modified by these factors [56,68]. It is notable that type III IFN (IFN-λ) is also generated via the TICAM-1 pathway in $CD8\alpha^+$ DC in mice and human $CD141^+$ DC in response to polyI:C [69]. Yet, in other cell types such as hepatocytes, the MAVS pathway participates in IFN-λ production [70].

In addition, tumor cells may liberate self mRNA, miRNA and other endogenous noncoding RNAs (**Table 2**), which become TLR3 ligands through conformational alterations which result in the formation of incomplete stems [71,72]. These self RNAs allow TLR3-positive host cells to induce IFNs and chemokines (**Figure 1B**). Once type I IFN and IFN-γ are robustly produced, the synergistic function of these IFNs results in the induction of IFN-stimulated genes (ISG) in the tumor and surrounding cells, including CXCL10 (IP-10) and CCL5 (RANTES) [72,73]. CXCR3 ligands (CXCL9, 10 and 11) are also expressed by these cells [73]. Since CXCR3 is mainly expressed on activated T and NK cells, these cytotoxic effectors converge upon the inflammatory nest, which includes the tumor microenvironment as well as secondary-affected organs. The tumor microenvironment is likely to be modified by these mediators in conjunction with cellular immune response.

These immunological aberrations may coincide with ecological environmental factors besides viral infection. Indeed, in mouse models, UV-B irradiation effects conformational changes in dermal mRNA to convert nonstimulatory mRNA to active TLR3 ligands by forming with incomplete stems [37], which then activate the TLR3 pathway, similar to virus-derived RNA [38], resulting in inflammatory sunburn. In any case, RNAs with bulged stems are functional as TLR3 agonists to induce IFN-α/β and possibly cellular immunity [37,38,66].

Whether endogenous TLR3 ligands are tumorigenic or tumoricidal remains to be determined. Necrosis-like cell death occurs in a cell type-specific manner as a result of death

signaling and liberates damage-associated molecular patterns (DAMP) of autologous TLR3 ligand (**Table 2**). Levels of RNA-derived TNF-α and its receptor, TNFR1, have been implicated in this process [74]. The RIP1/RIP3 complex, termed the necrosome, is responsible for switching between apoptosis and necroptosis [75,76]. TICAM-1 and RIP1 may be involved in the virus-derived as well as tumor cytolysis [77], although the possible involvement of RIG-I/MDA5 in cell death cannot be ruled out in some cases of viral infection [78]. DAMP and stemmed RNA can be liberated from tumor-infiltrating Mf as well as necroptotic tumor cells [77]. TNF-α and IL-6 are the pro-inflammatory cytokines released from Mf. A reported feature of exogenous dsRNA in the context of the tumor environment is to damage tumor cells by activation of Mf or the TLR3 pathway in these cells [10]. However, in tumor microenvironment containing tumor-infiltrating Mf, the role of the endogenous stemmed RNA in tumor progression and immune cell activation is the next issue to be elucidated (**Figure 2**).

7. Cellular immunity induced in tumor microenvironment

Once DC or Mf responds to an unusual innate dsRNA signature, cellular immunity is provoked against tumor cells with irregular modification of RNA-sensing pathways by these immune enhancers (**Figure 2**). NK cells and CTL are known to be associated with maturation of myeloid DCs after stimulation with dsRNA [79-81]. DCs express NK-activating ligands after recognizing dsRNA [82], and cell damage has been reported to play a role in the regulation of NK-activating ligands [83]. In this manner, dsRNAs are involved in tumor damage secondary to activation of cellular effectors. Subsequently, TLR3-stimulated DCs modulate cross-priming of CD8 CTLs through incorporation of dsRNA and Ag-mounted cell debris [84]. FasL and TRAIL are major effectors for the ligands of death receptors (DRs) [85].

Soluble mediators also function as effectors in response to dsRNA. The tumor microenvironment contains many cell types and tissues, on which dsRNA and DAMP act to effect the immune response (**Figure 2**). Systemic administration of polyI:C induces type I IFN and enhances local T-cell immune responses in the lung and liver [48,86]. It has been postulated that polyI:C-induced type I IFN mediates the production of IL-7 [88], which promotes T-cell-derived IFN-γ to enhance macrophage recruitment and CXCR3 ligand expression [86]. NK cells are involved in early onset of IFN-γ in response to polyI:C [32] after which IFN-γ is then robustly released through IL-7 production. IL-7 is produced in the lung and liver in a type-I IFN- and IFN-γ-dependent fashion [86,87]. In addition, polyI:C-induced IL-7 promotes expression of MCP-1, contributing to recruitment of macrophages and production of CXCR3 ligands by these cells [73,88,89]. This role of polyI:C in the tumor environment defines a new mechanism by which tumor-infiltrating T/NK cells boost local T-cell immunity and by which IL-7 bridges TLR3 signal to adaptive immunity.

Our laboratory has reported that a dsRNA analog strongly activates NK cells *in vivo* [32]. Two main routes for NK cell activation have been reported. Firstly, DCs secrete several cytokines, such as IL-12, IL-18, IL-15, and IFN-α/β in response to dsRNA, and these mediators act on NK cells [33,90]. Secondly, DCs express NK-activating ligands on their cell surface which activate NK cells through cell cell contact [57]. In mouse studies, transacting IL-15 and cell-surface NK-activating ligands are crucial in polyI:C-mediated NK cell activation [33,57]. The primary NK-activating ligand induced by polyI:C is IRF-3-inducing NK-activating molecule (INAM), which contributes to NK-sensitive tumor regression [57]. In a human system with BMDC and HCV-infected debris (a source of dsRNA), NK cells are activated by BMDC via the TLR3-TICAM-1 pathway in BMDC [82]. Based on these observations, INAM may therefore participate in dsRNA-derived NK activation. It is notable that the minimal dose of dsRNA for NK activation is higher than that required for induction of type I IFN in *in vivo* systemic administration studies.

RNA-derived molecular patterns of DAMP may cause TLR3-mediated inflammation resulting from physicochemical stimuli (**Figure 2**). However, the functional properties of stemmed RNA generated in tumor-related inflammation have not been well demonstrated [38]. Once antigens are presented on MHC class II in DCs upon internalization of tumor cell debris, CD4 T cells [91,92], including Th1, Th2, Th9, Th17, and Tregs, are driven in a context-dependent manner. Stemmed RNA and DAMP (**Table 2**) may act as modifiers of this event for CD4 T cells. The induction profiling of CD4 T-cell subsets critically affects the effector-inducing capacity of myeloid DC [91], although it remains unclear whether systemic type I IFN (and the MAVS pathway) is absolutely required or not for adaptive immunity. In addition, these stimulators may serve as the second signal of TLRs triggering DCs to induce cross-presentation, which leads to mounting Ag on MHC class I and subsequently induce the proliferation of CD8 T cells (CTL) [93]. Cross-presentation is enhanced by molecules such as type I IFN and CD40, and by immune cells, including CD4 T cells, NK cells, and NKT cells [93,94]. The mechanistic role of nucleic acids sensors in the presentation of exogenous Ag by DCs remains to be determined [61]. TLR3/TICAM-1 is the main pathway for inducing cross-presentation in response to dsRNA in DCs [34]. PolyI:C or virus dsRNA is an example of a TLR3 ligand, and the cross-presentation-inducing activity of these TLR3 agonists is noticeable if sufficient amounts of polyI:C are used [8]. While the effective adjuvancy of polyI:C has been reported by Steinman *et al.* [61,91,93], no report has definitively determined the dose of RNA sufficient to promote cross-presentation and latent cross-priming (CTL-inducing) ability in humans. Further therapeutic dose analysis will provide a basis for effective strategies of dsRNA

therapy in patients who do not respond to conventional cancer therapy.

8. Expert opinion

Here, we discussed the advantages of TLR3 agonists as a therapeutic potential against cancer. TLRs generally activate transcription factors, NF-κB, which closely associates with protumor activity, thereby application of TLR agonists to adjuvant immunotherapy for cancer treatment having been controversial. TLR3 is particular in the TLR family receptors because it is not involved in MyD88 activation but only in TICAM-1 for IRF-3/7 activation, which results in production of type I and III IFNs. TLR3 is localized to the endosomal membrane in mouse CD8α$^+$ DC and human CD141$^+$ DC, suggesting that in viral infection, DCs phagocytoze noninfectious dsRNAs liberated from infected dead cells together with viral antigens. TLR3 in the DCs senses the internalized dsRNA to signal the IFN-inducing pathway. Epithelial cells and fibroblasts express TLR3 on the cell surface and directly sample dsRNA outside the cells, which may reflect the role of TLR3 in testing environment around the cells. Similar events might happen in tumor cells and DC surrounding microenvironment. Expression of TLR3 is up-regulated during malignant transformation, by eIF2 and RB, suggesting that many tumor cells can be modulated by their own TLR3 signal. The RIP1/3 pathway downstream of TICAM-1 can induce NF-κB activation, apoptosis or necroptosis that facilitates liberation of tumor antigen and its uptake by DC. Necroptosis secondary to RIP1/3 signal may be a representative outcome induced by tumor cell TLR3, although the protumor activity that induces tumor progression via the TLR3/TICAM-1 pathway is predicted to be negligible compared to the MyD88 pathway. Besides TLR3, RIG-I and MDA5 act as cytoplasmic sensors to induce systemic cytokine/IFN production leading to high serum cytokine levels. The most prominent side-effect induced by dsRNA or polyI:C (or LC) is a life-threatening cytokine shock. Indeed, the serum cytokine/IFN levels in WT mice treated with polyI:C are highly increased, but the levels are kept low in MAVS-/- mice, suggesting that polyI:C-mediated cytokinemia is largely attributable to the MAVS pathway. Although serum cytokines are high in TICAM-1-/- mice, NK cell activation and CTL proliferation are severely impaired in the absence of TICAM-1. Ultimately, we would predict that exclusive stimulation of TLR3 (i.e., TICAM-1) does not allow the serum cytokine/IFN levels in mice, whereas cellular immune effectors NK and CTL are sufficiently driven by TLR3-directed immunotherapy even in MAVS-/- mice. The strategies for specific targeting of TLR3 in dendritic cells without affecting MDA5/RIG-I should be developed for more efficient antitumor immunotherapy. If TLR3-targeted dsRNA therapy is established, tumor regresses without evoking either tumor progression or cytokinemia, two major side-effects by dsRNA-mediated inflammation then being cleared. If these TLR3 outputs are reproducible in human patients with cancer, dsRNA derivatives specifically directed against TLR3 will be an excellent therapeutic candidate for tumor immunotherapy as an adjuvant.

Clinical studies of polyI:CLC therapy for cancer was started on 1985. Since then, many clinical trials have been performed with polyI:C or LC. Most of them suggested that low doses of polyI:C did not always bring the patients good prognosis. This suggests that low-dose administration of dsRNA to patients, which appears sufficient for induction of type I IFN, is insufficient for induction for DC-driven NK activation and CTL proliferation. If administration of high doses of harmless dsRNA is feasible for adjuvant therapy, then patients with cancer benefit from therapeutic use of dsRNA. Development of less-toxic compounds specific for TLR3 would help patients with inoperable or drug-resistant tumors.

Declaration of interest

This manuscript was sponsored by grants from the Japanese Government, particularly by MEXT Grant-in-Project 'the Carcinogenic Spiral' and the Japan Initiative for Global Research Network on Infectious Diseases (J-GRID). The authors declare no other conflicts of interest and have received no payment in preparation of this manuscript.