death of acinar cells. We agree with their statements that pancreatic fat accumulation is mainly induced by these two factors. In this study, pancreatic FI in cases represents any type of fat accumulation caused by any type of etiology. It has been reported that lipotoxicity caused by a high TG content induces inflammatory responses and necrosis in pancreatic acinar cells *in vitro*.[28,29] It has also been shown that c-Myc activity is required for growth and maturation of the exocrine pancreas and for the transdifferentiation of acinar cells into adipocytes in mice.[30] Thus, pancreas containing scattered adipocytes might be more sensitive to acinar cell damage due to lipotoxity and other genetic factors, and scattered FI may reflect the acinar cell death or transdifferentiation after the damage.

Some limitations could be pointed out in this study. The major limitation is that it lacked normal healthy controls because pancreatic sections could be obtained only from patients who had undergone pancreatoduodenectomy. A second limitation is that we could not measure FI in more than one pancreatic section, as areas for measuring FI were limited and small because the areas of tumor and secondary inflammation were avoided. Therefore, a future study using a non-invasive method will be required to evaluate FI in a large area/volume of pancreas from healthy and case subjects. Previously, we have reported a case of PDAC that was associated with marked FI in the pancreas, as seen on computed tomography images.[31] Computed tomography imaging of the pancreas would be a useful approach for accurate evaluation and follow-up of pancreatic FI in normal subjects, as well as in cohort studies. The third limitation is that we did not exclude the areas of pancreas with PanINs from the sections for measuring FI because it is known that PanINs are sometimes found in pancreatic tissue of the elderly, and also that a large number of PanINs with various grades are found in the pancreas of the patients with PDAC. Therefore, it is extremely difficult to measure FI in the pancreas tissue without PanINs, especially in the limited area for measuring FI. The fourth limitation is that BMI could be underestimated in the cases, because weight loss is a very common symptom of patients suffering from pancreatic cancer even though most cases were classified as stage IIA or IIB. The fifth limitation is that there is no validation study. To confirm the observation in the present study, the same study should be repeated with the same methods in another center (hospital/institution). The final limitation is that we cannot distinguish whether FI was a risk factor or a consequence of the cancer. The only way to demonstrate that FI is a risk factor for PDAC is to perform a prospective cohort study to observe whether individuals with fatty pancreas could develop PDAC. For this purpose, we are now trying to establish the methods to evaluate FI in a large area/volume of pancreas by non-invasive method, using computed tomography and magnetic resonance imaging. In addition, studies on pancreatic carcinogenesis using animal models of fatty pancreas would be helpful to elucidate underlying mechanisms.

In conclusion, there is a positive correlation between FI in the pancreas and pancreatic cancer. The development of effective detection methods and/or markers of FI, especially "fatty pancreas" with severe FI, is warranted for mass screening of individuals at high risk of pancreatic cancer at health examinations.

CONFLICT OF INTEREST

Guarantor of the article: Hitoshi Nakagama, MD, DMSc.

Specific author contributions: Mika Hori contributed to the design of the study, acquisition, analysis and interpretation of data, writing and drafting of the manuscript; Mami Takahashi contributed to the conception of the study, development of methodology and data analysis and revision of the manuscript; Nobuyoshi Hiraoka contributed to the histopathological analysis and revision of the manuscript; Taiki Yamaji contributed to the statistical analysis and revision of the manuscript; Michihiro Mutoh contributed to data analysis and revision of the manuscript; Rikako Ishigamori contributed to the histopathological analysis; Koh Furuta contributed to material supports in human serum analysis; Takuji Okusaka contributed to the clinical revision of the manuscript; Kazuaki Shimada contributed to the clinical revision of the manuscript; Tomoo Kosuge contributed to the clinical revision of the manuscript; Yae Kanai contributed to the histopathological analysis; Hitoshi Nakagama contributed to study supervision and revision of the manuscript.

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Study Highlights

WHAT IS CURRENT KNOWLEDGE

- Fatty infiltration (FI) in the pancreas is positively correlated with obesity and prevalence of DM.
- The association of FI in the pancreas with pancreatic ductal adenocarcinoma (PDAC) is unclear in humans.

WHAT IS NEW HERE

- FI in the pancreas is associated with PDAC development in humans.
- Body masss index (BMI) was identified as the most significantly associated factor with FI in the pancreas.
- FI in the pancreas may increase the risk of PDAC beyond the effect of obesity alone.

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DRによる大腸がん予防の有用性
ーアスピリンによる大腸ポリープ再発抑制ー

Potential of Drug Repositioning for Colorectal Cancer Prevention: Inhibition of Colorectal Polyp Recurrence by Aspirin

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有望ながん化学予防剤とその予防介入試験について，これまでの基礎資料を提示し，実現可能性の観点からドラッグ・リポジショニングが今後のがん予防に有用であることを述べる。具体的には，アスピリンのがん予防介入試験を題材にして，試験対象者数や研究デザイン，さらには政策への提言に関して考察する。

1. はじめに

「がん予防」というテーマは，一般になじみ深く，興味も高い。しかし我が国には，がん化学予防「薬」はない。我が国においても，多くの基礎研究の成果によりがん化学予防「剤」が見出されてきたのは事実であるが，がん予防「剤」が「薬」として実用化されるためには克服すべき問題が多く残っている。疾患名のない状態には原則として国民皆保険制度は適用されず，健康な状態を対象とした予防薬は保健の適用外と考えられている（そのため，胃がん予防に有効と考えられるピロリ菌の除菌は感染症由来の別疾患として保険適用を行い，また子宮頸がん予防に有効なHPVワクチンは保険適用でなく，公的補助のみである）。しかし，対象がしっかりと絞られており，エンドポイントもはっきりとした臨床試験における有効性を示せるデータがあれば，公的補助やオーファンドラッグ申請など，予防薬が公的に認められる将来像を描くことは十分に可能である。

日本人における真のがん予防を目指していくためには，日本人を対象とした予防介入試験による結果からの裏付けが必須であると言える。不十分とはいえ，ある程度の発がんメカニズムの解明も進み[1,2]，proof of conceptを担保しつつがん予防介入試験を行うことが可能である時代になってきた。したがって，日本人において実現可能性の高いがん予防介入試験に関する体制整備（基盤整備）を必要予算の概略を含めて提示していくことがこれからの重要な課題である。

筆者らはアスピリンを用いたがん予防臨床介入試験におけるアジアで初めての成功を本年発表した[3]。ランダム化比較試験（RCT）レベルのがん予防臨床試験は施行が難しく，その成果は，数十年に一度出るかというまれな出来事であり，今回の成果はがん化学予防剤開発のマイル・ストーンとなる成果であると自負している。食品成分ではなく合成化合物としては，1996年非環式レチノイドによる肝がん再発予防（岐阜大学　武藤教授）が我が国で初めて報告されたがん化学予防の

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成功例である[4]。この報告から今回の成功まで約18年の歳月が必要であったことを考えると実務的開発の難しさが想像される。

製薬企業は新薬開発に苦しんでいるが，この課題を克服する手段としてドラッグ・リポジショニング（DR）の概念がある。今回のアスピリン臨床試験はまさにその成功例であり，開発を促す追い風となると考えられる（探索レベルからがん予防剤を開発するのでは間に合わないほど，高齢化によりがん罹患者数は増加している）。医療経済的な意義を鑑みても，予防薬が公的に認められる可能性は十分にあると考えており，将来的ながん予防介入試験の成果からその実用への道筋を示せたら，企業に新たなフロンティアを示せ，日本発の薬剤開発成功例として，経済発展に大きく貢献できる可能性もある。そこで本稿では生活習慣に起因するがん種であり，日本人の罹患数の多いがんとして大腸がんを取り上げ，がん予防介入試験に向けた現状の整理と実現化に関して，アスピリン介入試験を中心に解説する。

2. 基礎研究において予防効果の確認されている大腸がん化学予防剤

1976年にM. Sporn博士ががん化学予防の定義（化合物を用いたがんの増殖抑制）を発表してから既に38年が経ち[5]，多くのがん化学予防剤の

表1　動物実験において大腸がん予防効果が確認されている主な物質（合成化合物）

カテゴリー		標的	化合物名
阻害剤	酵素	Carbonic anhydrase	Acetazolamide
		Cyclooxygenase	Aspirin
			Celecoxib
			JTE-523
			Indomethacin
			Mofezolac
			Nimesulide
			Piroxicam
			Sulindac
		Farnesyltransferase	Manumycin A
		HMG-CoA reductase	Pitavastatin
		iNOS	Aminoguadine
			L-NAME
			GOFA-L-NAME
			ONO-1714
			SG-51
		NADPH oxidase	Apocynin
		ODC	Difluoromethylornithine（DFMO）
		PAI-1	SK-116，SK-216
	受容体	Angiotensin I	Candesartan
			Losartan
		EGF	EKB-785
			Pelitinib/EKB-569
		Histamine	Cimetidine（H2）
		Prostaglandin E_2	ONO-8711（EP1）
			ONO-AE2-227（EP4）
活性化剤		AMPK	Metformin
		LPL	NO-1886
		PPAR	Pioglitazone

候補が挙げられた。しかし，臨床応用がされているものは乳がんに対するタモキシフェンなどごく少数に限られ，日本において臨床応用例はない。ここでは *Apc* 遺伝子変異動物や化学発がん実験系を含むげっ歯類の動物実験において，大腸発がん予防効果が確認されている合成低分子化合物および天然物をそれぞれ表1および表2にまとめてみた。

表2　動物実験において大腸がん予防効果が確認されている主な物質（天然物）

カテゴリー	化合物名等
Coffee	Chlorogenic acids
	Cafestol
Fat	Docosahexaenoic acid（DHA）
	Fish oil
Fiber	Cellulose
	Fructo-oligosacharide
	Guar gum
	Wheat bran
Phytochemical	Catechin
	Chafuroside
	Croein
	Curcumin
	Resveratrol
	Soy isoflavones
	Tea extract（green）
Vitamin	Folate（Vit. B）
Other	BCAA
	Bovine lactoferrin
	Magnesium
	Selenium
	Sphingomyelin-ceramides

2.1　大腸発がん予防効果が確認されている合成低分子化合物

合成化合物は大きく分子の阻害剤と活性化剤に分類される。一見して，活性化を促す低分子化合物は少なく，多くは阻害剤であることが分かる。また，阻害剤は酵素と受容体とを標的としている。疾患等のないヒトを対象としていることから，がん化学予防剤に求められる最も大切な条件として副作用がほとんどないことが求められる。抗がん剤に散見される DNA 合成阻害効果ではなく酵素活性阻害を標的としている候補物質が多いことは，そのことを物語っている。また，標的酵素の多くは炎症に関わり，標的受容体としては増殖因子に関わるものが多くを占めている。D. Hanahan 博士と R. A. Weinberg 博士が指摘しているように，発がんの初期段階において重要な役割を演じているのが，細胞増殖やアポトーシスに関わる分子である[1,2]。現在の分子生物学・遺伝学の進歩を受け，がん化学予防剤の開発も具体的分子を標的とした分子標的予防薬の開発が中心となってきていると言える。アスピリンなどのシクロオキシゲナーゼ（COX）の阻害（図1）やプロスタグランジンの受容体の阻害はメカニズム的に同様な増殖シグナルや抗アポトーシスの阻害を意味している。また，HMG-CoA 還元酵素阻害と farnesyl transferase 阻害もほぼ同様なメカニズム阻害に由来していると考えられる[6,7]。

2.2　大腸発がん予防効果が確認されている天然物

天然物としてがん予防効果が検討されている物

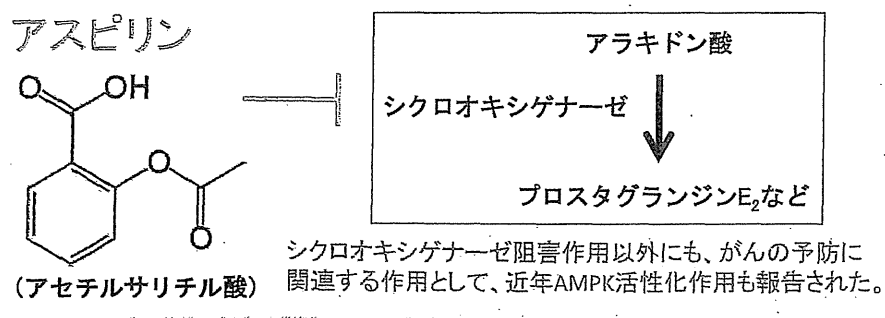

図1　アスピリンによるシクロオキシゲナーゼの阻害

質は，疫学研究によりその有効性が示唆されているものが中心である（表2）。発がんモデル動物を用いる実験によりその成分ごとの解析も行われている。例えばコーヒー成分であるクロロゲン酸，ω-3系脂肪酸であるドコサヘキサエン酸（DHA），フレンチパラドックスとして有名な赤ワイン成分レスベラトロールなどが有効成分として挙げられる。これらの想定作用機序は多岐に渡っており，多面的な効果が見出されている。しかしそれが逆に科学的な投与方法の確立やがん予防機構の解明を複雑にしているという一面がある。

3. RCTレベルで予防効果の確認されているがん化学予防剤

実際の介入試験に基礎データをつなげるためには多くの疫学的観察研究結果の蓄積が必要である。また，何にもまして優先順位の決定には，先にも述べたようにがん予防効果の強弱よりも副作用がないことが最優先されることを留意すべきである。そのような視点に立つと，介入試験に入る優先順位の高いがん化学予防剤の候補は，すでに市場に出ている薬剤の転用（DR）により開発するのが妥当と思われる。RCTレベルで大腸腺腫又は大腸がんの予防効果の報告されている主な物質を表3にまとめた。

表3　ランダム化比較試験にて大腸腺腫／がん予防効果が報告されている主な物質

カテゴリー	化合物名等	対象
合成化合物	Aspirin	Lynch症候群
		家族性大腸腺腫症
		大腸腺腫の既往者
	Celecoxib	家族性大腸腺腫症
	Sulindac	家族性大腸腺腫症
	DFMO＋Sulindac	大腸腺腫の既往者
天然物	Eicosapentaenoic acid（EPA）	家族性大腸腺腫症
	Bovine lactoferrin	大腸腺腫症

3.1　大腸発がん予防効果が確認されている合成低分子化合物

がん予防効果の確認されている薬剤を見ると，多くは抗炎症作用を主な機序としている。しかし，大腸がん予防薬として最も期待されていたコキシブ系薬剤（COX-2選択的阻害剤）に重篤な心血管系障害の副作用が見つかり，開発は振り出しに戻っていた[8]。その一方，アスピリンは世に出てから今年で115年目を迎え，副作用などが十分に分かっているため，近年再注目されている。本年公表した筆者らのデータではアスピリンは大腸腫瘍の再発リスクを40％も減弱させることを示唆するデータが得られた[3]。一方，糖尿病薬であるメトホルミンの大腸がんに対する予防効果は，十分に示されていない。その他，最近の臨床試験データベースを見ると，synthetic lethalな考え方を考慮して2剤以上の多剤併用投与が増えてきている。

3.2　大腸発がん予防効果が確認されている天然物

天然物としてはアジア人では魚油のエイコサペンタエン酸（EPA）の有効性が示されている[9]。ラクトフェリンに関しては動物実験ほどの強烈ながん予防効果がヒトを対象とした臨床試験では示されなかった[10]。その他，葉酸はがん高危険度群での検討はなく，13のRCTを集めたメタ解析でそのがん予防効果が否定されている。カルシウムやセレニウムの効果は報告により結果が異なり，レスベラトロールやクルクミンは安全性試験が終了，現在進行中の試験が多い。

4. 想定される段階別の臨床試験方法および対象集団

がんの予防をエンドポイントとした介入試験は主に欧米で行われており，我が国にはほとんどない。しかし，超高齢化社会を迎えて国民の医療への関心が高くなり，医療経済への対応が迫られる中，先制医療を目指した予防薬開発のニーズは高まってきている。そこで，がん化学予防剤のサイズ別の集団予防への適用性や予防介入試験のデザ

表4　想定される大腸がん化学予防剤の臨床試験のパターン

対象者数	対象者	デザイン	試験期間	目標とする政策	その他
1～数十人	遺伝性疾患等のがん高危険群 (lifetime risk 40-100%) 例）FAP，Lynch 症候群	症例検討 Phase I	—		企業参画のための エビデンス
～100 人	遺伝性疾患等のがん高危険群	RCT	半年程度	オーファン・ドラッグ 申請	
～数百人	がん中危険群 (lifetime risk 10-20%) 例）腺腫既往者／がんサバイバー	RCT	2 年程度	保険収載 保険適用拡大	診療ガイドライン への影響
～数千人 ～数万人	一般集団 (lifetime risk ＜10%)	単一介入	10 年程度	保険収載 保険適用拡大	

インを，アスピリンの臨床介入試験を例として以下に考察する（表4）。

4.1　がんの高危険群に対するがん予防介入試験

1人～数十人を対象とした症例検討では学術的なエビデンスは低いが，よりエビデンスの高い試験を施行するための，また企業との連携提案材料としての利用を考えて実施することができる。生涯に大腸がんに罹患する可能性により40～100%の集団をがんの高危険群と想定すると，家族性大腸腺腫症（FAP）患者やLynch症候群の患者が該当することになる。また，100人レベルではRCTの臨床試験を行うことができ，その試験期間も半年程度である[11]。しかし，逆に該当患者数も少なく，そのため全国規模の多施設の協力が必要となる（例えば，Juvenile polyposis，Cowden syndrome，Peutz-Jeghers syndromeなどは患者数が少なすぎて，RCTの施行は難しい）。一方，これらがんの高危険群に対するオーファンドラッグとしてのがん化学予防剤の申請は価値のあるものになると考えられる。

4.2　がんの中危険群に対するがん予防介入試験

生涯に大腸がんに罹患する可能性が10～20%の集団をがんの中危険群と想定すると，大腸腺腫の既往があり腺腫を取った患者や，大腸がん治療後のがんサバイバーにおける再発（3次予防の対象者）はその中危険群と考えられる。試験としては，数百人レベルでRCTを行うことができ，その試験期間も数年程度と考えられる。しかし，アスピリンの場合は，介入後に20年間観察すると介入後5年くらいから予防効果が強く見られてくることもあり[12]，試験期間が終了してもさらなる長期的観察が必要なこともあるため，計画を立てる時にはフェールセーフ的に考えておく必要がある。がんの中危険群に対するがん化学予防剤に対し，既知の薬からのDRでは，保険適用拡大を目指すことになると思われるが，予防剤は薬ではないので，何らかの疾患名をつけた保険適用拡大を狙うか，公的補助という別の方向性の可能性も考える必要がある。

4.3　一般集団に対するがん予防介入試験

一般化を目指すためには数万人レベルの臨床試験が必要となる。そのため薬剤の選定や対象者を考察すると，高齢化に伴う疾患（心血管疾患，痴呆等）や代謝疾患（糖尿病，脂質異常症，高血圧，骨粗鬆症など）など，薬適用者数の多い患者を対象とし，DRの考え方で利用できる薬剤を選定するのが現実的であろう。最近アスピリンの次に候補となるがん化学予防剤として，スタチン（脂質異常症治療薬），メトホルミン（糖尿病治療薬），ビスフォスフォネート（骨粗鬆症治療薬）が挙げられているが，この中でコホートデータが十分に

揃っているのはやはりスタチンである[13]。このレベルの試験のエンドポイントとしては複数想定されるが，大腸がんの発生にすると10年程度の試験期間が必要となる。集団における大腸がんの発生頻度からもRCTではなく多施設単一介入試験が選ばれる可能性が高い。対照をどこに設定するかの問題はあるが，十数年の服用とその結果がポジティブな場合，十分なエビデンスとして保険収載を含めた政策決定に影響を与えるものと思われる。

5. おわりに

私見ではあるが，DHAの基礎的動物データの報告年[14]と魚油の臨床介入試験の報告年[9]を考えると，臨床介入試験まで持っていくためには，現在においても約10年の年月が必要と推察される。また，割合を考えると動物試験で得られた100候補のうち数個がヒトでの臨床試験で検討できれば良い方と考えられ，RCTレベルまで検討できている薬剤は約40年の歴史の中でも海外を含めて数えるほどでしかない。臨床レベルに持っていける薬剤数もまだ十分ではなく，年間数例の有望ながん化学予防剤の候補物質が，動物レベル実験で精度良く実施・提案される必要がある。そしてこの選別を経た有力な候補物質を中規模な臨床介入試験で効果確認した上で大規模な臨床介入試験へと進めることが重要と考えられる。

「がん予防」の研究は，ビジネスとしての展開が開けていないことから，現時点では企業等の参入が難しい領域で，ほとんど全てのがん予防研究は公的研究費で行われている。直接的／実際的に役立つ結果をDRの観点より積み上げて，実務的な価値判断を一般消費者や行政当局に訴えて行く必要がある。がん予防研究推進のためにも世論喚起を引き続き行い，皆様のご協力を期待したい。

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REVIEW

Potential ability of xanthophylls to prevent obesity-associated cancer

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Abstract

Obesity-associated cancers, including colon cancer and breast cancer, are increasing in Asian countries with Westernized lifestyles as exemplified by reduced physical activity and increased fat/sugar consumption. An excessive accumulation of visceral adipose tissue causes insulin resistance, dyslipidemia and adipocytokine imbalance, and these factors are suggested to be involved in cancer promotion. To prevent obesity-associated cancers, researcher attention is increasing on the so-called "functional foods". In addition, new approaches to cancer control are in high demand, and using "functional foods" as supplemental or adjuvant agents in chemotherapy is thought to be a promising approach. One of these functional ingredients is xanthophylls, which are natural fat-soluble pigments found in fruits, vegetables, algae and other plants. Xanthophylls belong to the carotenoid class and have structures containing oxygen. Some studies have revealed that xanthophylls improve the inflammation status, serum triglyceride levels, blood pressure levels and liver function test values. Furthermore, recent studies show that xanthophylls possess high anti-cancer, anti-diabetic, anti-obesity and anti-oxidant properties. In this review, we highlight the recent findings for five xanthophylls, namely astaxanthin, β-cryptoxanthin, fucoxanthin, neoxanthin and zeaxanthin/lutein, and their relevance to cancer prevention.

Key words: Cancer prevention; Xanthophylls

Core tip: Xanthophylls belong to the class of carotenoids, and are natural fat-soluble pigments found in fruits, vegetables, algae and so on. It has been shown that the versatile functions of xanthophylls have great potential for the prevention of metabolic syndrome and cancers. Xanthophylls have proved safety, and several xanthophylls provide other health benefits, including improvement of inflammation, dyslipidemia, hypertension and liver function. These findings indicate that xanthophylls could be useful to prevent obesity-associated cancer.

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INTRODUCTION

Obesity has recently attracted much interest as a risk factor for several cancers, such as breast cancer and colorectal cancer[1,2]. Both metabolic syndrome that is characterized by obesity, hyperlipidemia, type 2 diabetes and hypertension

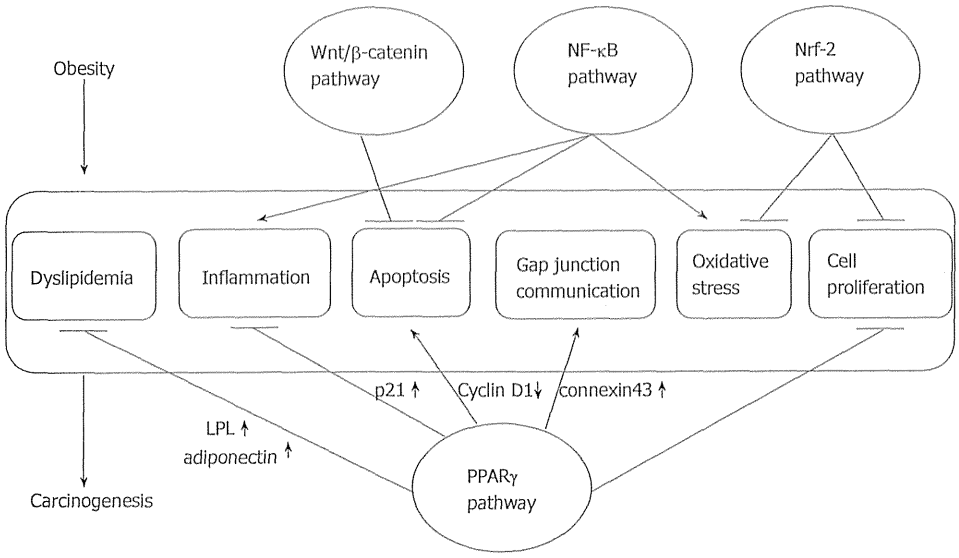

Figure 1 Possible mechanisms for obesity-associated cancer prevention. LPL: Lipoprotein lipase; NF-κB: Nuclear factor kappa B; Nrf2: Nuclear factor-erythroid 2 related factor 2; PPAR: Peroxisome proliferator-activated receptor.

Table 1 Obesity-associated cancers

Type of cancer
Breast (postmenopausal)
Colorectum
Endometrium
Esophagus
Gallbladder
Kidney
Pancreas
Thyroid

and obesity-associated cancers (Table 1) are extremely common in Western countries, and they are currently increasing in Eastern countries, including Japan. The factors linking obesity and cancer are becoming apparent, and they are insulin resistance, dyslipidemia and a subsequent adipocytokine imbalance (Figure 1)[1,2]. Carotenoid intake is reported to be inversely associated with obesity and with the risk of many cancers[3-6].

Carotenoids are fat-soluble pigments found in fruits, vegetables, algae and other plants. Humans cannot synthesize carotenoids, and we should therefore consume them as part of our diet. Carotenoids belong to the tetraterpenoid category, and they can be divided into xanthophylls and carotenes according to whether the structure contains oxygen or not. Carotenoids with structures containing oxygen are xanthophylls. As the name indicates, the color of xanthophylls is usually yellow, and they are usually lipophilic because of the long unsaturated aliphatic chain in their structure.

Because conventional chemotherapy has failed to reduce the mortality rates of common cancers, including obesity-associated cancers, new approaches to controlling the development of cancer are in great demand[7]. One approach is the use of functional foods/plant-derived agents as supplemental or adjuvant agents in chemotherapy[8,9]. Another approach is chemoprevention for the control of cancer development[8,9]. In both methods, using xanthophylls seems to be an attractive approach. As shown in this review, xanthophylls provided health benefits, such as improvements in inflammation, dyslipidemia, hypertension and liver function. Moreover, the biological significance of xanthophylls as important candidates for the chemoprevention of cancer is becoming clearer, and the safety of xanthophylls has been affirmed, as described in this review. Another candidate called - carotene is the most abundant dietary carotenoid, and long-term supplementation with this compound has been shown to be ineffective for cancer chemoprevention in several recent large-scale intervention trials[10-12].

In this review, we focus on recent findings for five xanthophylls as follows: astaxanthin, β-cryptoxanthin, fucoxanthin, neoxanthin and zeaxanthin/lutein, and their relevance to cancer prevention (Figure 2).

ASTAXANTHIN

Distribution and nature of astaxanthin

Astaxanthin (AX) is a natural fat-soluble red pigment and belongs to the xanthophyll subclass of carotenoids. Dietary sources of AX are eggs of salmon and trout, skin of red sea bream, crabs, shrimps and lobsters. AX is synthesized in microalgae (*Chlorella zofingiensis, Chlorococcum* and *Haematococcus pluvialis*). Krills (*Euphausia superba*) feed on the microalgae and in turn are fed upon by fishes. The microalga, *H. pluvialis*, is the main source of natural AX and is able to accumulate up to 4%AX on dry weight basis[13-15]. AX extracted from *H. pluviali* is used as a food dye in many countries. AX exists in stereoisomers and geometric isomers. *H. pluvialis* biosynthesizes the (3*S*, 3'*S*)-isomer, meanwhile *P. rhodozyma* biosynthesizes the (3*R*, 3'*R*)-isomer. AX has two hydroxyl groups and is able to react with fatty acids and proteins. AX is found as free, mono- and di-ester forms in organisms[13].

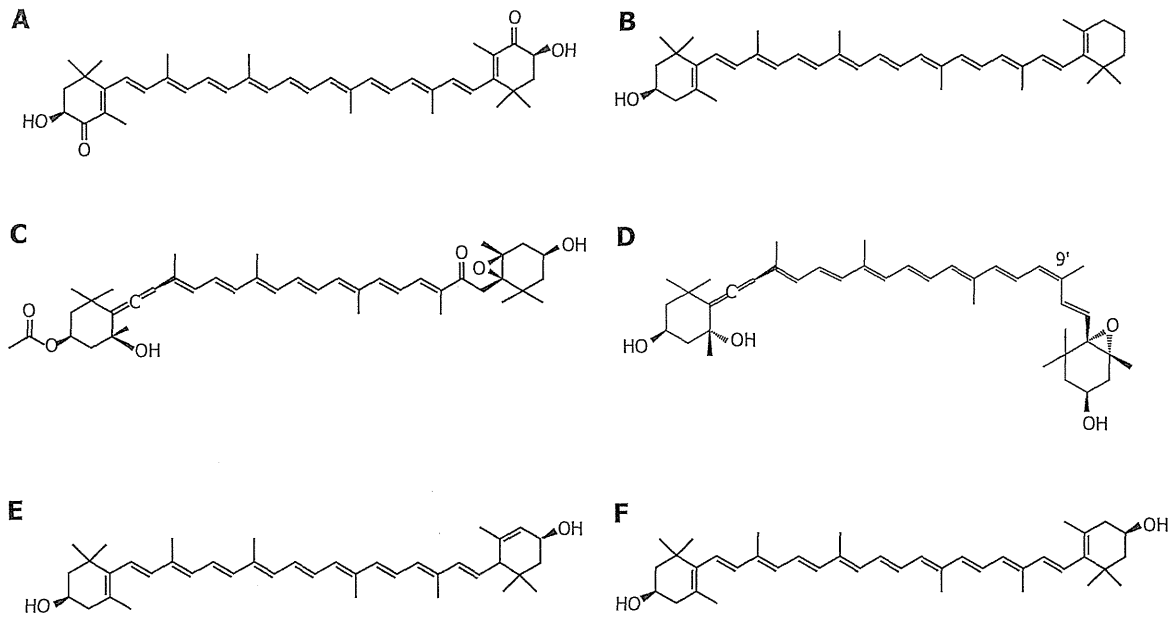

Figure 2 Structure of xanthophylls. A: Astaxanthin; B: β-cryptoxanthin; C: Fucoxanthin; D: 9'-cis-neoxanthin; E: Lutein; F: Zeaxanthin.

AX can take to transverse cell membrane orientation, and shows strong antioxidative activity[13,15]. After oral administration of AX, AX changes to all-*E*, 9*Z*-, 13*Z*-geometrical isomers and 3*R*,3'*R*-, 3*R*,3'*S* meso-, 3*S*,3'*S*-optical isomers, all of which can be detected in human blood[16].

Safety profile

Many experimental and clinical studies have demonstrated the safety of AX[13,17]. In a subchronic toxicity study in rats, feeding AX-rich microalgae biomass corresponding to doses of 465 and 557 mg AX/kg per day for 90 d in male and female rats, respectively, revealed no adverse events[18]. A randomized, double-blind, placebo-controlled study has demonstrated that it is safe to administrate 6 mg/d AX in healthy adults for 8 wk[19], and a significant decrease of triglycerides and increase of adiponectin and high density lipoprotein cholesterol in participants with mild dyslipidemia by administration of AX at doses of 12 mg/d and 18 mg/d for 12 wk[20].

Preclinical studies and anti-cancer mechanisms

Oxidative stress and inflammation are closely related to carcinogenesis (Figure 1), and many antioxidants, including carotenoids have been demonstrated to decrease cancer development in experimental animal models[14]. There are papers on preventive effects of AX on urinary bladder[21], oral[22,23], and colorectal[24,25] carcinogenesis. In mouse urinary bladder cancer induced by N-butyl-N(4-hydroxybutyl)nitrosamine (OH-BBN), AX administration at a dose of 50 ppm in water for 20 wk after OH-BBN exposure for 20 wk resulted in a decrease in the incidences of precancerous lesions and bladder cancer[21]. In rat oral carcinogenesis induced by 4-nitroquinoline 1-oxide (4-NQO), the incidence of oral precancerous lesions in rats treated with 20 ppm 4-NQO and 100 ppm AX was smaller compared to those of the non-treatment group, and oral neoplasms did not observed in rats fed AX among the 4-NQO exposure[22]. In these studies, AX decreased cell growth activity in the non-cancerous epithelial tissues of 4-NQO-exposed animals[21,22]. AX has also been demonstrated to show preventive effects in 7,12-dimethylbenz[a]anthracene (DMBA)-induced hamster buccal pouch carcinogenesis *via* nuclear factor-erythroid 2 related factor 2 (Nrf2) activation[23]. Moreover, AX has been shown to inhibit nuclear factor kappa B (NF-κB) and Wnt/β-catenin signaling pathways[26]. Related to colorectal carcinogenesis, AX at 500 ppm in diet significantly decreased the development of aberrant crypt foci (ACF) and the incidence and multiplicity of colorectal tumors induced by azoxymethane (AOM)[24]. AX at 200 ppm in diet also suppressed mucosal ulcers induced by dextran sulfate sodium (DSS), and development of dysplastic ACF and colonic adenocarcinoma induced by both treatment of DSS and AOM[25]. In addition, AX reduced the number and size of aflatoxin B1-induced liver preneoplastic foci in rats[27]. Growth of WAZ-2T cells, mammary tumor cells, inoculated into the mice mammary fat pad was also inhibited by AX at 100 ppm or 400 ppm in diet[28]. Lipid peroxidation activity in tumors was reduced in tumors treated with 400 ppm[28]. AX markedly attenuated the promotion of hepatic metastasis of P815 mastocytoma cells in a syngenic graft model under restraint stress[29]. In *in vitro* cell culture systems, AX suppressed invasion of rat ascites hepatoma AH109A cells[30]. AX inhibited cell proliferation and decreased cell viability of leukemia K562 cells *via* induction of apoptosis along with up-regulation of peroxisome proliferator-activated receptor (PPAR)γ and p21, and down-regulation

of cyclin D1 [31]. Induction of connexin 43, gap junction protein, through activation of PPARγ is suggested to be one of the anti-tumor mechanisms of AX[32,33]. Up-regulation of the Nrf2 pathway is also involved in antioxidant activity of AX[23,31,34], and may improve mitochondrial function[35]. However, the role of Nrf2 activation in anti-tumorigenesis is controversial. The oncogenic *K-ras* gene induces Nrf2 expression, and detoxification of reactive oxygen species promotes tumor growth[36]. Deficiency of Nrf2 has been reported to increase induction of tumors in urethane-induced mouse lung carcinogenesis, but reduce the number of malignant tumors harboring activated mutation in the *K-ras* gene, indicating that Nrf2 prevents initiation but accelerates progression under the activation of the K-ras signaling pathway[37]. Indeed, there is a report that effects of AX differ at the stages of initiation and the stage of promotion in mammary tumors. AX fed before tumor initiation delayed mammary tumor growth and modulated immune response, but AX supplementation after tumor initiation resulted in more rapid tumor growth[38]. Thus, use of antioxidants for cancer prevention is considered to be useful at the time before tumor initiation, but more caution is required in using them after the stage accompanied with activated K-ras signaling.

Clinical studies

A randomized, double-blind, placebo-controlled study has demonstrated that AX reduces oxidation of fatty acids[39], decreases oxidative stress markers[40] and inflammation[41], and improves dyslipidemia[20] and age-related dysfunction of eyes[42,43] and brain[44]. However, human cancer prevention studies using AX have not yet been reported.

β-CRYPTOXANTHIN

Distribution and nature of β-cryptoxanthin

β-cryptoxanthin (β-CRX) is one of the naturally occurring carotenoid pigments, and is also classified as a xanthophyll. Its unique character is that it is found in specific fruits and vegetables such as mango, papaya, loquat, Japanese persimmon, peach, sweet red peppers and citrus fruits of the mandarin family[45,46]. Satsuma mandarin, *Citrus unshiu*, is one of the most β-CRX rich fruits in Japan. The content of β-CRX in *C. unshiu* reaches several mg/100g wet weight. The level of β-CRX in Valencia orange is very low and grapefruit has been found to be devoid of it.

In the human body, β-CRX is easily converted to vitamin A (retinol) and is therefore considered as a provitamin A. It is also known that β-CRX might be easily absorbed[47], and is accumulated in various organs[48]. Moreover, it can be stored for several months in the human body[49]. Serum β-CRX concentration could be around 96 μg/dL[50]. It is also reported that β-CRX concentration in Japanese mother's milk and serum are nearly parallel with their intake of the Satsuma mandarin, and are higher than other countries[51,52].

Epidemiologic studies

Many epidemiological studies showed the intake of β-CRX was significantly associated with reduced risks of type 2 diabetes [relative risk (RR) = 0.58][53] and rheumatoid arthritis (RR = 0.59)[54]. β-CRX supplementation significantly decreased cigarette smoke-induced lung squamous metaplasia and inflammation[55]. Regarding cancer risk, several observational epidemiologic studies suggest that β-CRX could potentially prevent cancer development. The demonstrated cancer risks for lung, esophageal and bladder were 0.76 (RR), 0.16 [odds ratio (OR)] and 0.74 (RR), respectively, comparing the highest to lowest quintile of intake[56-58]. A greater intake of β-CRX was also inversely associated with developing undetermined cervical atypical squamous cells (OR = 0.4)[59]. Interestingly, the serum level of β-CRX is lower in the patients of liver cancer than that in healthy counterparts[60]. These results suggest that a high serum β-CRX concentration or intake of β-CRX is beneficial to human health.

Safety profile

The scientific panel on additives and products or substances used in animal feed (FEEDAP) panel members considered β-CRX to appear not to be mutagenic and show no clastogenic activity[61]. In subchronic studies, The FEEDAP panel could not find any adverse effects[61]. Also an acceptable daily intake has not been determined[61]. Previously, we have reported the chemoprevention effect of β-CRX against chemically-induced bladder carcinogenesis in ICR mice[62]. Mice were fed with 1, 5 and 25 ppm of β-CRX for 24 wk, and no clinical signs of toxicity and poor condition, low survival or histopathological changes were found[62]. Many epidemiological studies[53-60,63-68] indicated that administration of β-CRX is safe for human health.

Preclinical studies and anti-cancer mechanisms

Various functions of β-CRX have been reported recently. β-CRX is an antioxidant phytochemical and may help prevent oxidative damage[69]. Thus, it is believed that β-CRX has health benefits for people with risk of chronic diseases.

Numerous possible mechanisms for the anti-carcinogenic potential of β-CRX have been proposed. These include the antioxidant function that is associated with the enhancement of DNA repair[55,69], suppression of efficacy of key proinflammatory cytokine expression, such as tumor necrosis factor-α[55] and an apoptotic induction effect[70]. Also, β-CRX is known to stimulate the expression of the *RB* gene (a tumor-suppressor gene) and *p73* gene (a *p53*-related gene)[71] and reduce the expression of NF-κB and activator protein-1 (AP-1), that induces numerous genes including inflammation, cell proliferation, and apoptosis[55]. These mechanisms indicate that β-CRX may be a promising chemopreventive agent against cancer. Indeed, β-CRX exerts an anti-tumor promoter action *in vitro*[72] and

inhibits chemically induced carcinogenesis *in vivo*[62,71,73,74]. Previously, we investigated the effects of β-CRX extracted from *C. unshiu* oranges on OH-BBN-induced urinary bladder carcinogenesis in male ICR mice[62]. OH-BBN-exposed mice were fed with 1, 5 and 25 ppm of β-CRX for 24 wk starting 1 wk after the cessation of OH-BBN exposure. Feeding with β-CRX decreased the incidence and multiplicity of precancerous and cancerous urinary bladder lesions. Especially, 25 ppm β-CRX markedly reduced the occurrence of bladder cancer. Meanwhile, β-CRX is also reported to reduce mouse skin[71], mouse lung[74] and rat colon[71] carcinogenesis. In our report, β-CRX lowered ratios of cyclin D1-positive cell in various urinary bladder lesions, meaning that reduction in the incidence of precancerous and cancerous urinary bladder lesions is due to reduced cell cycle progression[62].

Clinical studies

The efficacy of β-CRX supplementation on obesity have been investigated[75]. Seventeen postmenopausal obese women were provided 200 mL of a beverage containing β-CRX (1.56 mg/serving and 4.7 mg/d) for 3 wk[75]. As a result, the levels of serum β-CRX were significantly elevated from 0.28 (initial period) to 1.15 mg/mL, and high molecular weight-adiponectin was also elevated from 9.8 to 11.1 mg/mL[75]. At the end of the study, the levels of serum triglyceride ($P = 0.057$) and total plasminogen activator inhibitor-1 (PAI-1) ($P = 0.052$) tended to decrease. Nishino *et al*[60] reported an intervention study where β-CRX-rich mandarin orange juice (3 mg β-CRX in 80 mL) was provided for 12 wk to obese men or obese men with elevated serum γ-glutamyl transpeptidase (γGTP) levels[60]. After drinking β-CRX for 12 wk, body weight ($P < 0.001$), BMI ($P < 0.001$) and β-GTP levels ($P < 0.005$) were decreased.

An intervention trial regarding prevention of liver cancer has also been reported[60]. Viral hepatitis with cirrhosis patients were randomly assigned into two groups in the trial. The treatment group was administered mandarin orange juice enriched with β-CRX and with the carotenoids mixture (lycopene, β-carotene and α-carotene). Patients in the control group were administered a carotenoids mixture alone. At year 2.5, cumulative incidence of liver cancer/hepatocellular carcinoma development in the mandarin orange juice group was lower than that of the carotenoids mixture alone group ($P = 0.05$). The combinational use of natural carotenoids containing β-CRX might be valuable for the prevention of liver cancer in hepatitis virus infected patients with cirrhosis.

FUCOXANTHIN

Distribution and nature of fucoxanthin

Brown seaweeds include *Undaria pinnatifida* (wakame), *Hizikia fusiforme* (hijiki), *Laminaria japonica* (ma-kombu) and *Sargassum fulvellum*. The Japanese have been estimated to intake wakame at 1 g/d[76]. Brown seaweeds are known to contain many bioactive components, *i.e.*, fucoxanthin (FX), fucoidan, vitamins, minerals, dietary fibers, proteins, ω-3 polyunsaturated fatty acids (PUFAs), polysaccharides, other carotenoids and various functional polyphenols. Fucoidan is a sulfated polysaccharide that is one of the major bioactive components in seaweed[77,78], but we would like to focus on FX in this review. FX is a xanthophyll belonging to non-provitamin A carotenoids, constructed with an unusual allenic bond, an epoxide group, and a conjugated carbonyl group in a polyene chain[79]. Some reports demonstrated that the FX content of *U. pinnatifida* is approximately 1.0-3.0 mg/g dry weight through one life cycle[80,81]. It has been proven that mice convert FX into keto-carotenoids by oxidation of the secondary hydroxyl groups (FX + H_2O → FuOH; FuOH + NAD^+ → amarouciaxanthin A + NADH)[79]. On the other hand, oral administration of kombu extract containing FX in humans revealed that the FuOH and the *cis*-isomer of FuOH could be found in the serum, detected by HPLC[82].

Safety profile

FX has been proved to be safe with no side effects by single (1000 or 2000 mg/kg BW) and repeated (500 or 1000 mg/kg BW for 30 d) oral dose toxicity studies in male and female mice[83]. In the repeated doses study, histological examination of the gonadal tissues, kidneys, liver and spleen revealed no abnormal changes[83]. In rats, 13-wk oral subchronic toxicity studies suggested that more than 2000 mg/kg BW of microalgal FX oil induce the 50% lethal[84].

Preclinical studies and anti-cancer mechanisms

Many studies suggested FX possesses anti-cancer potential, especially shown in colon cancer cell lines (Caco-2, DLD-1 and HT-29), liver cell lines (HepG2), prostate cancer cell lines (DU 145, LNCaP and PC-3) and urinary bladder[85-88]. The main biomolecules involved in anticancer mechanism is assumed to be the biomolecules related to apoptosis and cell cycle[89,90] and those may associate with antioxidant activity through their free radical scavenging action[91]. Moreover, inhibition of PI3K/Akt and NF-κB signals were reported in human cervical and breast cancer cells, respectively[92,93].

Its metabolite fucoxanthinol (FuOH) also has inhibitory effects on cancer cell growth[94,95], and 1,2-dimethylhydrazine-induced formation of colonic ACF in mice and AOM/DSS-induced colon carcinogenesis[25,96]. To find new cancer prevention approaches, we investigated the combination effect of FuOH and 1α,25-dihydroxyvitamin D_3 (1α,25$(OH)_2D_3$), and found inhibition of cell viability and induction of apoptosis in DLD-1 and HT-29 cells[97]. Down-regulation of PPARγ and NF-κB p52 were suggested to be involved in the inhibition of cell viability due to the combination of FuOH and 1α,25$(OH)_2D_3$. It has been shown that activation of PPARγ suppresses intestinal polyp development in *Apc*-mutant mice and AOM-induced colonic ACF development in obese KK-*A*^{y} mice[98,99].

Clinical studies

FX has been reported to provide health benefits in humans, such as improvement of obesity, reduction of inflammation, healthy triglyceride levels, and improvements in blood pressure levels[100,101].

After daily intake of *U. pinnatifida*, FuOH is detectable in human plasma[82]. Although metabolites of FX could be measured as a marker of exposure, effects of FX or FuOH in human carcinogenesis have not been reported to date. From the aspect of obesity-associated cancer, we here introduce one study that has been conducted to assess the effects of FX supplementation on weight loss. FX supplementation on obese patients with nonalcoholic fatty liver disease results in the improvement of liver inflammatory markers, such as alanine aminotransferase, aspartate aminotransferase, C-reactive protein, γ-glutamyltransferase (γGT, GGT)[101]. Of note, it has been demonstrated that increased of GGT plasma levels are associated with an increased risk of pancreatic cancer[102,103], nevertheless GGT has no causative role itself.

It is also interesting to mention that intake of 5 g/d *U. pinnatifida* stimulated a significant 50% reduction in urinary urokinase-like plasminogen activator receptor (uPAR) proteins in postmenopausal women. uPAR, is the membrane receptor for uPA, responsible for extracellular membrane proteins degradation and PAI-1, responsible for the inhibition of plasminogen activation[104]. Generally, uPAR is known to be higher in postmenopausal women as well as in breast cancer patients[105]. Moreover, it has been reported that uPA and/or PAI-1 is positively correlated with poor prognosis in patients with breast cancer, *i.e.*, correlation with cancer metastatic potential[106,107]. Thus, uPA, PAI-1and uPAR might be used as prognostic markers for breast cancer[108], and FX may reduce such a tumor marker.

NEOXANTHIN

Distribution and nature of Neoxanthin

Neoxanthin (NX), a non-provitamin A carotenoid, has an unusual allenic bond and a 5,6-monoepoxide as well as FX. NX is widely present in terrestrial and marine biota and the occurrence of two geometric *cis/trans* isomers is known to be species dependent[109-111]. The 9'-*cis* form of NX (9'-*cis* NX) is mainly localized and used in the photosynthetic organs of spinach leaves and marine algae such as *Euglenophyta*. It is also used as a precursor of abscisic acid, a plant hormone[112,113]. Whereas the all-*trans* form of NX is predominant in the petals of globeflower and yellow rose, this xanthophyll is not involved in the photosynthetic system[111,114]. We mainly obtain the 9'-*cis* NX from leafy green vegetables. Fresh spinach contains 9'-*cis* NX around 5 mg/100 g in fresh leaf[110]. It has been estimated that 9'-*cis* NX exists at 0.95 μmol/L in digested fluid (9 L/d), when we ingest 100 g/d spinach.

The 5,6-monoepoxide moiety in 9'-*cis* NX is easily isomerized to 5,8-epoxide under the acidic conditions of the stomach and generates almost equal amounts of (8'-*R*/*S*)-neochrome[115,116]. After a 1-wk spinach intervention (3 mg 9'-*cis* NX/day), highly hydrophilic xanthophylls of 9'-*cis* NX and (8'-*R* and 8'-*S*)-neochromes appeared at a very low level in human plasma (about 1 nmol/L)[117]. It is known that the uptake of various carotenoids by human colon cancer cells (Caco-2 cells) positively correlates with the lipophilicity of the carotenoids[118]. The highly hydrophilic xanthophylls such as NX, FX and violaxanthin could be detected slightly in human plasma, when we intake purified forms and food matrices[79,101,117,119-121]. Because of the poor intestinal absorption of NX, a considerable amount of ingested 9'-*cis* NX and (8'-*R* and 8'-*S*)-neochrome would be delivered to the colon, and even if absorbed in the small intestine, they would be metabolized easily.

Preclinical studies and anti-cancer mechanisms

It has been reported that both 9'-*cis* NX and all-*trans* NX possess strong potential of cell growth inhibition and apoptosis induction in human prostate cancer cells[87,94,115,122], human colon cancer[122-124], mouse melanoma[122] and mouse embryonic mesenchymal cells[125]. In addition, several researchers have reported that 9'-*cis* NX, all-*trans* NX and (8'*R*/*S*)-neochrome have cancer preventive effects[126], and also anti-tumor promoter functions[70]. Moreover, induction of cell cycle arrest[115], anti-oxidant properties[127] and anti-obesity properties[128] have been reported. Recently, we additionally demonstrated that 9'-*cis* NX rapidly accumulated in the mitochondria, caused mitochondria $\Delta\Psi$ loss and thereafter the release of cytochrome *c* and production of apoptosis-inducing factor in human colon cancer cells[123]. It is regrettable that there is little information about the anti-cancer mechanisms of dietary NX in mammals, except for that described above.

Safety profile and clinical studies

No safety profile and clinical studies have been reported on 9'-*cis*NX, any -*trans* NX and (8'*R*/*S*)-neochromes. However, epidemiological data show that higher intake of fruits and vegetables, rich in highly hydrophilic epoxyxanthophylls such as NX, is associated with a lower risk of colorectal cancer[129,130]. Further studies are required to elucidate the clinical beneficial properties of NX.

ZEAXANTHIN/LUTEIN

Distribution and nature of zeaxanthin / lutein

Zeaxanthin (ZX) and lutein also belong to the xanthophyll family. Their unique character is that they are the only carotenoid among more than 600 species of carotenoid existing in eye tissue, especially in the retina[131]. Lutein can be photochemically transformed to meso-ZX. They are stereoisomer of each other, differ by the location of a double bond. Lutein is abundant in egg yolk, and in dark-green leafy vegetables, such as broccoli, brussels sprouts, kale and spinach[132]. In the human body, lutein is distributed at the skin, breasts, cervix uteri, and also found in serum in high amounts. Serum lutein and

ZX levels are reported to be around 180 and 20 ng/mL, respectively[133]. They are assumed to play a critical role in ocular health because they act as strong anti-oxidants and filtered out high-energy blue light[134]. Of note, no correlation between plasma concentrations of lutein/zeaxanthin and BMI or insulin resistance has been reported[135].

Epidemiologic studies

In many papers, target organs for lutein are reported to be the eyeballs, the skin and the heart. Regarding ocular conditions, age-related macular degeneration, cataracts, and retina pigmentosa have been reported to have some correlation with lutein. Lutein also possesses a preventive function of cardiovascular diseases/stroke[131,134,136,137].

Regarding lung cancer, some epidemiologic studies state lutein has an important cancer preventive function[4,14]. A ten-year study of 120000 United States people revealed that lung cancer incidence was significantly reduced in those who ingested a high amount of total carotenoids, including lutein and ZX[138]. Similar relationships were found in Fijians, when compared to the other South Pacific islands' people. Fijians intake 25 mg lutein daily on an average (200 g dark greens), whereas other 20 South Pacific countries intake less lutein in diets[139]. Thus, there was a clear inverse association with lutein intake and lung cancer incidence.

Regarding colorectal cancer, inverse associations with dietary lutein intake have been reported[124], and serum ZX concentration by Okuyama *et al*[140]. However, no association has been detected between the levels of plasma lutein and the risk of gastric cancer[141].

Regarding skin cancer risk, the specific effects of lutein are not fully known. The only reported data is that a combination of carotenoids may protect erythema development in human skin[142], and that may be correlated with the presence of skin cancer or precancerous lesions[124].

Regarding breast cancer, there is some possibility for protective effects of lutein[6,14,143]. Intake of lutein-rich foods significantly lowered the risk of premenopausal breast cancer. The Nurse's Health Study demonstrated a weak inverse association, but significant, between lutein and ZX intake and the breast cancer risk among premenopausal women[6]. Of note, the protective effect of lutein and ZX was strongest in patients have a family history of breast cancer. Also there is a report that increasing serum levels of lutein and ZX were associated with a reduced breast cancer risk, but the trend was only marginally significant in a case-control study[143]. There is a report comparing biopsy samples from breast cancer tissue and benign mammary tissue. In this report, increasing lutein and ZX concentrations tends to decrease the risk of breast cancer[144]. Meanwhile, Other studies have shown that there are no differences of lutein and ZX concentrations in mammary adipose tissue between benign breast tumors and breast cancer[145]. New York University Women's Health Study, a nested case-control prospective study, demonstrated an inverse relationship between plasma levels of lutein, but not ZX, and risk of breast cancer[146].

Regarding other cancers, significant inverse relations were observed for lutein and ZX in oral cavity and pharyngeal cancer[147].

Safety profile

No toxicities or adverse reactions for intake of lutein/ZX have been reported at doses up to 40 mg/d for 2 mo[131,148]. High doses of β-carotene supplements (> 30 mg/d) are well known to be associated with carotenodermia[149], and the same could happen when we consume high doses of lutein and ZX. Also it has been demonstrated that lutein has no mutagenic effect in the Ames test[150].

Preclinical study and anticancer mechanism

Lutein/ZX is thought to have a superior anti-oxidant ability to scavenge free radicals than other carotenoids. An *in vitro* study showed that lutein could quench peroxy radicals and play a guarding role against oxidative injury[151,152]. In this experiment, a synergistic antioxidant effect was obtained with a combination of lutein and lycopene[153]. Carotenoids also show a superb function for immune response[154].

Lutein could also function as an anti-carcinogenic reagent, such as a modulator of cell growth and apoptosis signaling. Lutein induces cell cycle arrest in human prostate and esophageal cancer cell[155,156]. Lutein induces apoptosis in transformed cancer cells but do not induce apoptosis in normal human mammary cells through modulating the ratio of Bcl-xL/Bax protein expression[157]. Meanwhile, ZX, structural isomer of lutein, induced cell cycle arrest in human beast cancer cells[158]. Lutein stimulates some genes involved in T-cell transformations activated by antigens, cytokines and mitogens[159]. Lutein interacts with carcinogens such as 1-nitropyrene and aflatoxin B1, and lowered its carcinogenetic activity[150,160]. In a recent report, female BALB/c mice were fed a diet containing lutein for 14 d, and then inoculated with 0 to 2.5 × 10^5 mammary tumor cells. The results demonstrated that 0.002% and 0.02% lutein lowered both mammary tumor incidence and tumor growth[161].

FUTURE ASPECTS

The versatile functions of xanthophylls have shown great potential for the prevention of metabolic syndrome and cancers, both *in vitro* and *in vivo*. Xanthophylls have been verified as safe with no side events, and several xanthophylls provide other health benefits, including improvements in inflammation, dyslipidemia, hypertension and liver function, as shown in this review. The accumulated evidence indicates the functionality of xanthophylls as anti-obesity and anti-insulin-resistance functional foods, implying that xanthophylls could be useful in preventing obesity-associated cancer.

The chemical synthesis of each xanthophyll is not impossible, but it may be very expensive. However, the

promising results obtained from *in vivo* studies encourage researchers to undertake more clinical studies in humans. We have some information about xanthophylls trials, and we should further promote human clinical studies to obtain information about the adequate dosage of xanthophylls needed to prevent cancers.

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