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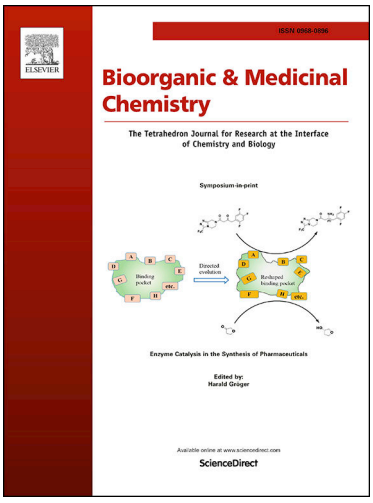

Disease-associated acrolein: A possible diagnostic and therapeutic substrate for in vivo synthetic chemistry

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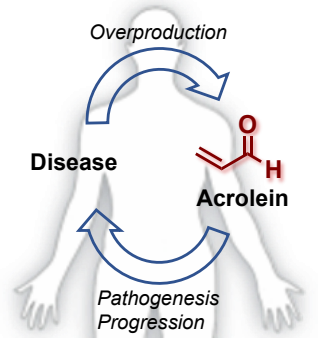

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ABSTRACT

Acrolein, a highly reactive α,β-unsaturated aldehyde, is a compound to which humans are exposed in many different situations and often causes various human diseases. This paper summarizes the reports over the past twenty-five years regarding disease-associated acrolein detected in clinical patients and the role acrolein plays in various diseases. In several diseases, it was found that the increased acrolein acts as a pathogenetic factor. Thus, we propose the utility of over-produced acrolein as a substrate for a promising therapeutic or diagnostic method applicable to a wide range of diseases based on an in vivo synthetic chemistry strategy.

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Acrolein is a highly electrophilic α,β-unsaturated aldehyde (Figure 1) to which humans are exposed in many situations when it is produced by environmental sources such as engine exhaust, cigarette smoke, and vapors of over-heated cooking oil. Acrolein is formed during the combustion of organic substances.[1,2] It has also been reported that the ordinary intake of foods and beverages such as fruits, vegetables, alcohol, and milk includes ppm levels of acrolein exposure.[3,4] In particular, acrolein is generated during the cooking processes of fermentation or heating via the Maillard reaction.

In the human body, acrolein can be endogenously produced through the enzyme-mediated oxidation of polyamines[5] or threonine[6] and is also generated by the oxidation of unsaturated fatty acids with reactive oxygen species (ROS).[7] Polyamines are known to be essential for cell growth and exist mainly as RNA-polyamine complex in cells. Once RNA is degraded by ROS or cell death, polyamines are released in their free form. Free spermine, a representative polyamine, is metabolized by spermine oxidase (SMO) producing spermidine and 3-aminopropanal, which spontaneously decomposes to ammonia and acrolein (Figure 2A). Threonine, which exists at up to 200 μM in plasma,[8] is oxidized by hypochlorous acid that is produced by myeloperoxidase (MPO), and further reactions including decarbonation and dehydration produce acrolein (Figure 2B).[6] Since MPO is released by the activation of phagocytes, acrolein is produced in high concentrations at sites of inflammation. Another source of acrolein is from unsaturated fatty acids via oxidation with ROS.[7] One production scheme from arachidonic acid, which includes peroxidation and β-cleavage, is shown in Figure 2C. However, the oxidative lipid degradation is quite complex, so that acrolein is not the exclusive product; the reaction also includes the production of malondialdehyde, crotonaldehyde, 4-hydroxynonenal (4-HNE), and others. Perhaps due to this complexity, the amount of acrolein produced from lipid oxidation is lower compared to polyamines.[9] Based on these mechanisms, acrolein levels in oxidatively stressed cells sometimes reach the millimolar scale.

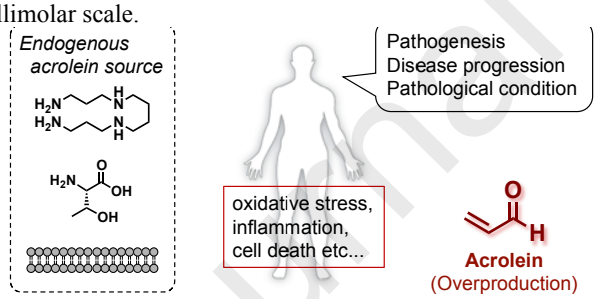

Figure 1. Schematic illustration of disease-associated acrolein.

Chemically, acrolein is highly reactive and has become recognized as a real hazard to human health. The toxicity and function of acrolein are well-summarized in some reviews.[2,10-12] In particular, the intrinsic reactivities in biological settings often lead to many problematic alkylations of proteins or nucleobases by reacting mainly with amino, thiol, and hydroxyl groups. Indeed, acrolein is known to be more toxic to cells than ROS[13] such as H_2O_2 or hydroxyl radicals, which are major oxidative stress factors associated with a variety of disorders, and inhibitors of several specific enzymes such as glucose and glutamate transporters.[14] Therefore, it is considered that acrolein is associated with many kinds of diseases and pathologies.

Recently, some clinical studies have investigated and analyzed the presence of endogenous acrolein or its metabolites in diseases (Table 1) and its influence on pathological conditions. Acrolein is upregulated in many diseases through the effects of oxidative

diagnosis and pathological staging, as well as treatments. This review summarizes the reports available regarding disease-associated acrolein which has been detected in clinical patients over the past 25 years. We also highlight the utility of acrolein as a substrate for therapy or diagnosis on the basis of an in vivo synthetic chemistry strategy.

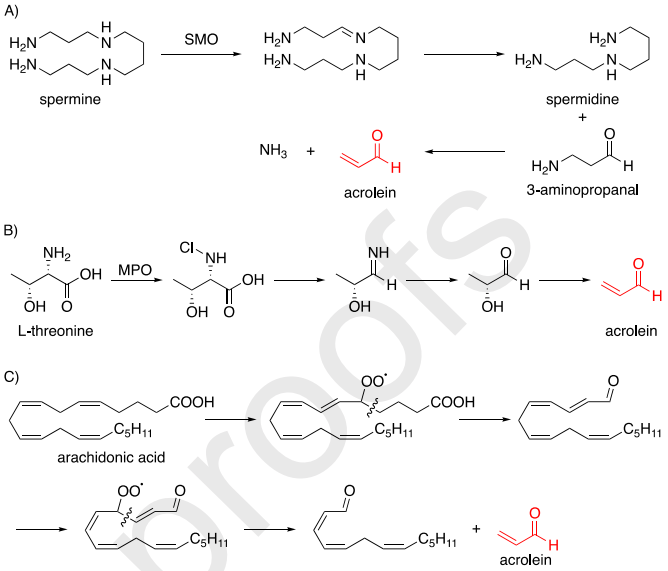

Figure 2. Endogenous acrolein production from A) spermine, B) threonine, and C) arachidonic acid.

2. Intrinsic reactivity of acrolein for detection and in vivo synthetic chemistry

Acrolein shows various reactivities owing to both the α,β-unsaturated carbonyl and aldehyde structures. In the human body, through the reaction of two acrolein molecules with a Lys residue in the protein, 3-formyl-3,4-dehydropiperidine (FDP)-Lys[15] is produced via a doubly conjugated intermediate (Figure 3, arrow 1). FDP-Lys is gradually converted to 3-methylpyridinium (MP)-Lys over an extended period of time.[16-18] As a result of the reaction with the guanine base in DNA, acrolein-guanine adducts (Acr-dG) are generated as cyclic products with two possible isomers (Figure 3, arrow 2).[19] On the other hand, the acrolein-glutathione (GSH) adduct also obtained by a Michael addition of a thiol group of GSH (Figure 3, arrow 3) subsequently undergoes cleavage of amide bond and acetylation via the mercapturic acid pathway to generate 3-oxopropylmercapturic acid (OPMA).[20] Reduction or oxidation of OPMA produces 3-hydroxypropylmercapturic acid (HPMA) or carboxyethylmercapturic acid (CEMA), respectively. An 8-membered ring compound is also produced by the reaction with Lys or polyamines (Figure 3, arrow 4).[21] Moreover, endogenous acrolein can react with some administered compounds in vivo. For example, additional thiol compounds, such as *N*-acetylcysteine (NAC) and 2-mercaptoethanesulfonate (Mesna), can capture acrolein via a Michael reaction (Figure 3, arrow 5). Hydrazine or hydroxylamine compounds, such as hydralazine and *N*-benzylhydroxylamine, are also effectively trapped in the aldehyde structure of acrolein (Figure 3, arrow 6).[22,23] Furthermore, a highly substrate-specific [2+3] cycloaddition between acrolein and phenyl azide provides an unstable 1,2,3-triazoline compound without a catalyst (Figure 3, arrow 7).[24] It is noteworthy that these reactions are found under in vivo conditions.

On the other hand, acrolein was derivatized to some detectable compounds, on the basis of its unique reactivity, by pre-treatment

of p… aldehyde structure can change to stable hydrazone or oxime by treatment with 2,4-dinitrophenyl (DNP) hydrazine[25] and *O*-pentafluorobenzyl (*O*-PFB) hydroxylamine.[6] In the presence of ammonium acetate, two molecules of 5,5'-dimethyl-1,3-cyclohexanedione (DCHD)[26] react with acrolein to produce the tricyclic compound by a Hantzsch reaction. In addition, the reaction with 3-aminophenol under reflux conditions generates 7-hydroyquinoline, which shows fluorescence emission.[27]

3. Disease-associated acrolein produced in patients

3.1. Sjögren's syndrome

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disorder mainly affecting the salivary and lacrimal glands.[28] The cardinal symptom is dry mouth and eyes as a result of decreased salivary and lacrimal secretions caused by the destruction of these glands. Although the etiology is not clearly understood, autoantibodies recognizing Sjögren's syndrome A (SSA) and Sjögren's syndrome B (SSB) were frequently found in the sera of pSS patients.

Two clinical studies were performed for female patients with pSS and healthy women or women with dry eyes and/or dry mouth as control (Table 1).[29,30] The concentration of FDP-Lys, in particular as albumin or immunoglobulin-conjugated acrolein, in saliva and plasma was measured by ELISA or western blotting. The FDP-Lys level was significantly increased in saliva from patients with pSS compared to controls, while there was no difference in plasma FDP-Lys. In addition, FDP-Lys in saliva was inversely correlated with the flow rate of saliva, which was used as an index of the severity of salivary gland destruction.

The activities of autoantibodies recognizing SSA and SSB in saliva from patients with pSS were approximately 3- to 5-fold higher than those from control subjects.[30] Interestingly, the activity of the autoantibodies increased with the acrolein treatment of saliva from control subjects. MS/MS analysis of immunoglobulins from patients with pSS showed some acrolein conjugation with cysteine, histidine, and lysine residues, of which modification would affect the antigen recognition ability. Thus, the changed antigen recognition ability of immunoglobulins due to acrolein conjugation is at least partially associated with autoimmune diseases.

3.2. Rheumatoid arthritis

…often involving two kinds of autoantibodies, rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibody (ACPA). Although the pathogenesis of RA is not completely understood, it is known that the arginine residue in protein is changed to citrulline by peptidylarginine deiminases, which are recognized by ACPA as causing the immune response. In addition, several epidemiological studies have proposed an association between RA and oxidative stress.[31]

Plasma FDP-Lys levels in patients with RA and normal subjects were measured by ELISA (Table 1).[32] The RA group showed significantly higher FDP-Lys levels than the control group. The FDP-Lys level increased even in patients with early RA without any inflammatory findings, and there is no correlation between the FDP-Lys level and disease duration and/or degree of inflammation. In addition, other clinical studies revealed that the

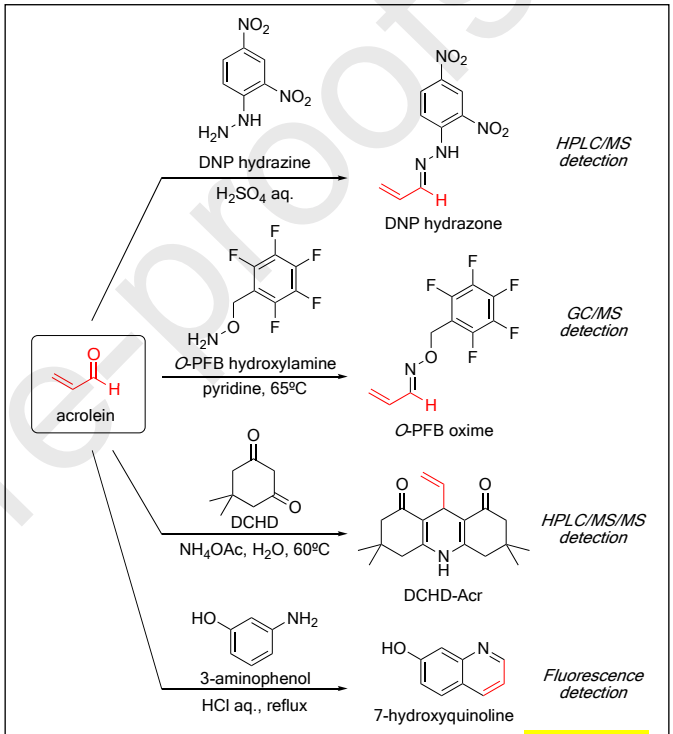

Figure 4. Derivatizations of acrolein in vitro (under non-physiological conditions) to analyze the acrolein level of specimen from clinical patient. The structure derived from acrolein is shown in red.

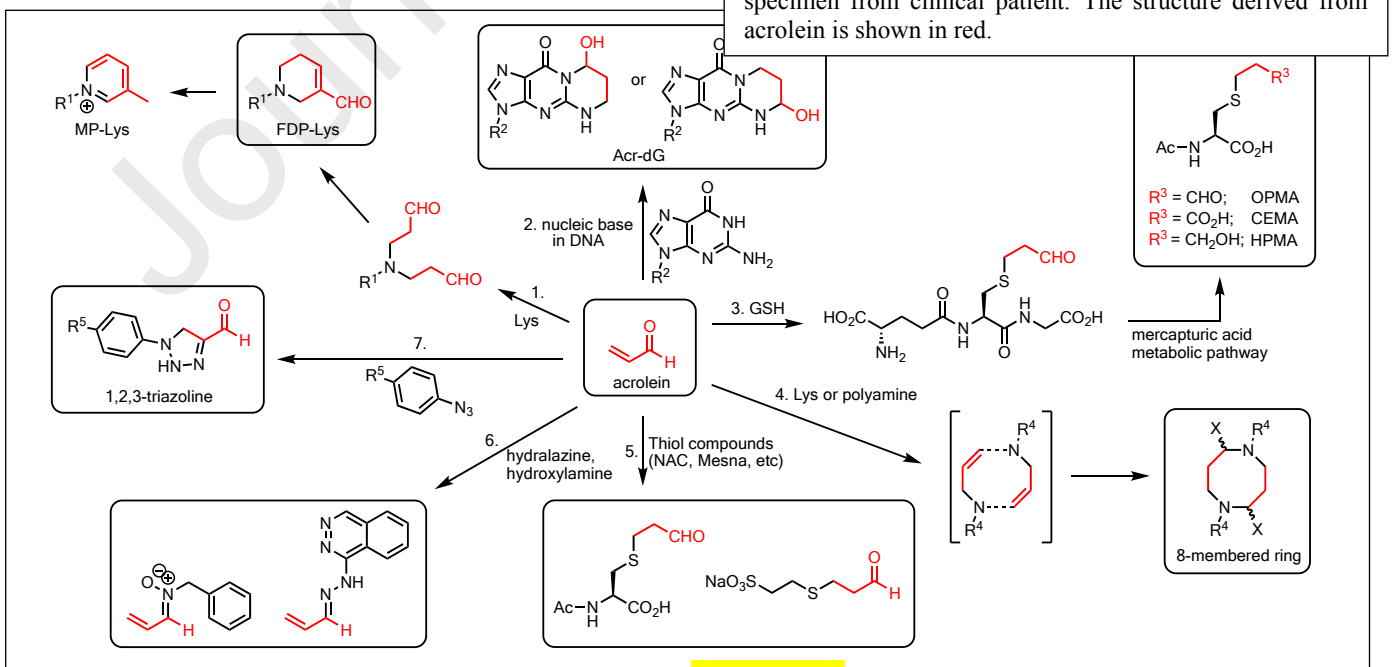

Figure 3. Acrolein-related reactions which proceed under in vivo (physiological) conditions. The structure derived from acrolein is shown in red.

leve
acrolein, significantly increased in the plasma and urine of patients with RA.[33,34] These clinical studies suggest that the relationship between lipid peroxidation and the development of RA and FDP-Lys levels could be applied in the diagnosis of early RA. Moreover, smoking history was associated with a high value of ACPF and RF in patients with RA,[35] and acrolein is possibly one of the pathogenetic factors in RA, similar to its role in pSS.

3.3. Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is a common, complex disorder that is characterized by progressive airflow obstruction due to varying degrees of bronchitis and emphysema.[36] It is thought that cigarette smoke including high levels of acrolein is involved in the pathogenesis of COPD. Acrolein could induce neutrophil activation, resulting in enhanced systemic acrolein production, as acrolein can absorb via lung tissue due to its membrane permeability.

For clinical measurements of acrolein in COPD, plasma and homogenized lung tissue were obtained from patients with COPD, current smokers without COPD, and non-smokers.[37] Acrolein concentrations in the supernatants of homogenized tissue were measured by HPLC/MS analysis after derivatization of acrolein by DNP hydrazine. As predicted, acrolein was expressed strongly and differently in the lung tissues of patients with COPD and non-COPD smokers compared to non-smokers, presumably due to direct exposure to acrolein from cigarette smoke. On the other hand, plasma concentrations of acrolein were significantly higher in patients with COPD than in non-COPD smokers. Therefore, the increased acrolein levels in plasma may be derived not only from cigarette smoke but also endogenous acrolein production and would be associated with the pathology of COPD through interference in the balance of oxidative stress versus antioxidant potentiality.

3.4. Acute alcoholic hepatitis

Excessive intake of alcoholic beverages leads to acute alcoholic hepatitis (AAH) which involves increased inflammation, oxidative stress, lipid peroxidation, and hepatocytes death,[38] although the exact mechanisms remain unclear. It is known that free radicals generated by alcohol consumption and metabolism can result in oxidative stress, leading to the production of acrolein by peroxidation of unsaturated fatty acids.

Urinary HPMA was assessed by UPLC/MS in patients with severe or non-severe AAH and healthy subjects.[39] The HPMA levels in patients with severe AAH were increased compared to healthy controls, but the non-severe cases showed similar results with controls. In addition, the involvement of acrolein in liver injury is supported by the correlation between hepatocyte death markers and HPMA combined with proinflammatory cytokines, which contribute to hepatic injury. Indeed, the protective effects of hydralazine, a known anti-hypertensive drug and acrolein scavenger (Figure 3, arrow 6), for hepatic cells was proven in animal models.[40] Thus, the HPMA level can be used for diagnosis of disease severity in AAH and treatment assessment in severe ALD. Acrolein scavenging has therapeutic potential for liver injury in ALD.

Table 1. List of clinical investigations of disease-associated acrolein.

Disease	Detected acrolein product	Detection method	Specimen	Ref
pSS	FDP-Lys	Western blotting, ELISA	saliva, plasma	29, 30
RA	FDP-Lys	ELISA	plasma	32
	acrolein (O-PFB-oxime)	GC/MS	plasma, urine	33
	(DNP-hydrazone)		homogenized lung tissues	37
AAH	HPMA	UPLC/MS	urine	39
DM	FDP-Lys	ELISA	urine	42, 43
	HPMA, CEMA	UPLC/MS	urine	44
AD	acrolein (DCHD-Acr)	HPLC/MS/MS	homogenized brain tissue	48
	acrolein(O-PFB-oxime)	GC/MS	homogenized brain tissue	49
	FDP-Lys	Dot-blotting	homogenized brain tissue	49
	FDP-Lys	ELISA, Western blotting	plasma, cerebrospinal fluid	50, 51, 53
	HPMA	HPLC/MS/MS	urine	52, 53
Stroke	FDP-Lys	ELISA	plasma	54, 55
	HPMA	HPLC/MS/MS	urine	55, 56
	MP-Lys	ELISA	plasma	17
SBI	FDP-Lys	ELISA	plasma	59
Renal failure	FDP-Lys	ELISA	plasma	62
	Acrolein(7-hydroxy-quinoline)	Fluorescence measurement	plasma	62
Tumor	Acr-dG	Dot-blotting	tissue DNA	63
	FDP-Lys	ELISA	plasma	63
	HPMA	LC/MS/MS	urine	63
	FDP-Lys	Immunohistoch-emical staining	tissue	64
	Acrolein	Fluorescence microscope	tissue	67

3.5. Diabetes mellitus

Diabetes mellitus (DM) is a metabolic disorder that induces hyperglycemia through the breakdown of the blood glucose regulatory system for various reasons. There are a number of hypotheses of its etiology which are intricately connected.[41] DM is classified as two types, type 1 and type 2, based on the failure mode of the blood glucose regulatory mechanism. In both types, blood glucose is not stored as glycogen in the liver or adipose tissue, leading to hyperglycemia. It is known that the metabolism of polyol, advanced glycation end products, and the TCA (tricarboxylic acid) cycle induce oxidative stress and are a common pathology of DM.

Clinically, an association between both types of DM and acrolein metabolites has been reported by several studies. FDP-Lys from the urine of patients with type 1 or type 2 DM was measured by ELISA (Table 1).[42,43] FDP-Lys levels in both studies were significantly increased compared to control subjects. The other study showed an association of the other urinary metabolites, HPMA and CEMA, with type 2 DM and insulin resistance.[44] Furthermore, it was suggested that acrolein has been associated with the dysregulation of glucose transport in human endothelial cells, diabetic nephropathy,[45] and diabetic retinopathy.[46] Accumulation of FDP-Lys involved the abnormalization of Müller glial cells occurring in the early stages of diabetic retinopathy.[46]

3.6. Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive impairment of cognitive function.

The ease mechanism is still unclear. The accumulation of amyloid β peptide (Aβ) in the brain and increased oxidative damage is a process thought to be involved in the development of AD.[47] Before AD is identified symptomatically, Aβ begins to aggregate. This stage is referred to as preclinical AD (PCAD), and the disease progresses to AD via mild cognitive impairment (MCI).

The acrolein level in homogenized brain tissues [hippocampus/parahippocampal gyrus (HPG), superior and middle temporal gyrus (SMTG), and cerebellum (CER)] of MCI, early AD, and control subjects were analyzed by LC/MS/MS after derivatization with DCHD.[48] Acrolein levels in all three brain regions of patients with early AD and MCI were significantly increased in comparison with control subjects, while there were no statistical differences between MCI and early AD. More recently, the levels of FDP-Lys and extractable acrolein in brain tissues (HPG, SMTG, and CER) from patients with PCAD were evaluated by dot-blotting and GC/MS, respectively.[49] The extractable acrolein in the HPG of patients with PCAD significantly increased compared to control subjects, contrary to the decrease in CER. There were no significant differences in the levels of FDP-Lys in the HPG, SMTG, or CER of patients with PCAD compared to control subjects.

To evaluate the utility of acrolein metabolites for AD diagnosis, FDP-Lys levels in plasma[50] or cerebrospinal fluid[51] of patients with AD were evaluated by ELISA or western blotting, because homogenized brain tissue was not suitable as a diagnostic specimen. FDP-Lys levels were increased in patients with AD and MCI compared to control subjects. In contrast, other studies showed that urinary HPMA was decreased in patients with AD and MCI compared to the control,[52,53] which is likely attributed to the depletion of GSH. These studies cannot distinguish MCI and AD in patients solely by acrolein metabolite levels.

Moreover, we reported that acrolein smoothly reacts with polyamines, such as spermidine and spermine, to obtain an 8-membered ring compound (Figure 3, arrow 4) that can suppress the toxic aggregation of Aβ (Figure 5).[21] This reaction is likely an innate biological defense mechanism of polyamines and could possibly be applied as a therapeutic strategy against AD through the utilization of acrolein as a treatment modality.

3.7. Stroke

Stroke, including cerebral infarction (CI) and cerebral hemorrhage (CH), is a sudden focal neurologic deficit caused by vascular insult involving cell damage in the central nervous system (CNS). Neurons contain high concentrations of polyamines, which act as neuromodulators and are perturbed after cerebral ischemia.[54] In addition, the activity of polyamine oxidases is considered to be upregulated by cell damage in the CNS.

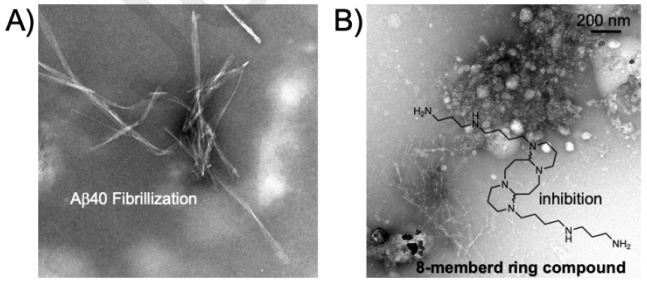

Figure 5. Fibrillization of Aβ40 peptide in the A) absence or B) presence of an 8-membered ring compound.

Plasma FDP-Lys levels of patients who have suffered a stroke were analyzed by ELISA (Table 1),[55,56] and the levels were increased compared to control subjects. While MP-Lys in albumin severity and prognosis of patients who have suffered a stroke. In contrast, urinary HPMA was decreased in patients compared to control subjects, due to the depletion of GSH, which is supported by the decrease of plasma GSH levels in patients.[56,57] The change of HPMA in patients is found in both types of stroke, CI and CH, with no statistical difference.[57] A mechanistic study suggested that the acrolein is produced via activation of the NF-kB pathway during ischemia,[56] and acrolein elicited a vicious cycling of oxidative stress resulting in stroke-related neuronal damage. Therefore, acrolein has been suggested as the primary culprit of neuronal damage in patients who have suffered a stroke. Of note, NAC or *N*-benzylhydroxylamine effectively prevented neurotoxicity by scavenging toxic acrolein in an animal model (Figure 3, arrow 5 and 6).[56,58]

Increased acrolein production was not only seen in severe strokes but also silent brain infarctions (SBI).[59] Measurements of FDP-Lys in the plasma of SBI and normal subjects revealed that the levels were significantly higher in patients with SBI. Analysis of FDP-Lys levels along with other factors including interleukin-6 (IL-6), C-reactive protein (CRP), and age can predict SBI with 89% sensitivity and 91% specificity in a receiver operating characteristic curve.

3.8. Tumor

A tumor is a proliferative lesion that invades surrounding normal tissue and is often fatal. Cancer cells are known to utilize ROS,[60] such as H_2O_2, and polyamines[61] to drive proliferation and other events required for tumor development. Therefore, cancer cells are believed to produce a significant amount of acrolein associated with a high concentration of an acrolein source, polyamines, and conditions conducive to oxidative stress.

3.8.1. Urothelial carcinomas from chronic kidney disease

It is known that urothelial carcinomas (UC) are highly prevalent in patients with end-stage renal disease. Patients with chronic kidney disease (CKD) have high plasma concentrations of polyamines, as their ability to excrete these substances is decreased. Along with this, free acrolein and FDP-Lys levels in plasma have been found to be increased in patients with renal failure.[62] In patients with concomitant UC and CKD, plasma FDP-Lys and urinary HPMA levels have been found to be significantly increased compared to those of control subjects.[63] Moreover, Acr-dG levels in tumor tissues are statistically correlated with CKD stages in patients with UC. Furthermore, several gene mutations have been identified in the extracted p53 gene, a tumor suppressor gene, from patients with UC. These results indicate that acrolein acts as an endogenous uremic toxin and carcinogenic factor via DNA damage and would contribute to UC formation in patients with CKD.

3.8.2. Colon cancer

Immunohistochemical distributions of FDP-Lys and p53 protein in colon tumors were evaluated using their respective monoclonal antibodies (MAbs) (Table 1).[64] These investigations showed that the FDP-Lys levels increased along with the incidence of colon carcinogenesis. Levels were found to be moderate in tubular and villotubular low-grade adenomas and abundant and diffuse in high-grade villotubular adenomas and Dukes A carcinomas. FDP-Lys levels in cancer gradually decreased as colon carcinomas progressed (Dukes B and C), although FDP-Lys was found to be abundant in the non-malignant colon epithelium of these patients. While there was no relationship between acrolein adducts, FDP-Lys, and p53 distribution with respect to protein levels, acrolein seems to be associated with the transition from benign to malignant colon tumors.

Acrolein seems to be over-produced in tumors, but the analytical method used thus far involving acrolein or acrolein metabolites requires several specimen pre-treatment steps and is time-intensive. Moreover, these values would not reflect the exact acrolein level in real time for various reasons including the slow formation of FDP-Lys.[65] Therefore, a novel analytical method is required in order to apply acrolein as a diagnostic substrate during the intraoperative pathological diagnosis of the tumor.

We have reported on the specific and rapid reactivity of acrolein with phenyl azide to give the unstable 4-formyl-1,2,3-triazoline derivative (Figure 3, arrow 7), which spontaneously decomposes to a diazo compound.[24] To apply this reaction as a chemical probe for imaging of cancer, the TAMRA fluorophore is attached to the phenyl azide, referred to as the click-to-sense probe (CTS probe, Figure 6A). The probe can be internalized into cells; if the CTS probe subsequently encounters intracellular acrolein, the 1,3-dipolar cycloaddition and further tandem reactions proceed and the resultant diazo intermediate reacts with the nearest organelle to anchor the fluorophore via a covalent bond (Figure 6B).[66] The unreacted probe can be washed out to reduce the background fluorescence as the probe has the ability to be shuttled inside and outside of the cell. This reaction achieves the visualization of cancer in live cells even at nanomolar levels of acrolein. Herein, we propose a concept of "Transformative In vivo Tandem reaction (TIT reaction)", that efficiently converts the reactive in vivo substances (such as acrolein) into certain bioactive, diagnostic or therapeutic molecules through the tandem reactions, directly in cells, plants, animals or human patients.

cancer tissues (Table 1).[67] The resected tissue specimens were derived from cancerous tissue [invasive ductal carcinoma (IDC) and ductal carcinoma in situ (DCIS)], normal breast gland (NBG), and ductal hyperplasia (DH) from patients with breast cancer who underwent breast-conserving surgery. The fluorescence images are shown in Figure 6C. The fluorescence intensity of tissues from cancers (IDC and DCIS) were statistically higher than that from non-cancerous NBG and DH, indicating that cancerous (IDC and DCIS) tissue could be distinguished from non-cancerous tissue (NBG and DH) with high specificity and sensitivity. Importantly, the CTS probe labeled the cancer tissues with similar fluorescence intensity, regardless of phenotype status such as estrogen receptor or human epidermal growth factor receptor 2. Moreover, the fluorescent images of cancers are in agreement with those of H&E staining (comparison of Figure 6C and 6D). Thus, this clinical study succeeded in visualizing cancer morphology in live tissue by utilizing cancerous acrolein as a diagnostic substrate.

4. Perspective of disease-associated acrolein as a diagnostic and therapeutic substrate

Acrolein and its metabolites were detected in many kinds of diseases associated with oxidative stress, which sometimes express complicated pathologies due to the local and systemic influence of oxidative stress. Analysis of endogenously produced acrolein would help to understand the unclear pathogeneses of these diseases. Moreover, acrolein is considered to be a potent substrate for use in the diagnosis or treatment of various diseases, owing to the disease-specific overproduction of acrolein. In

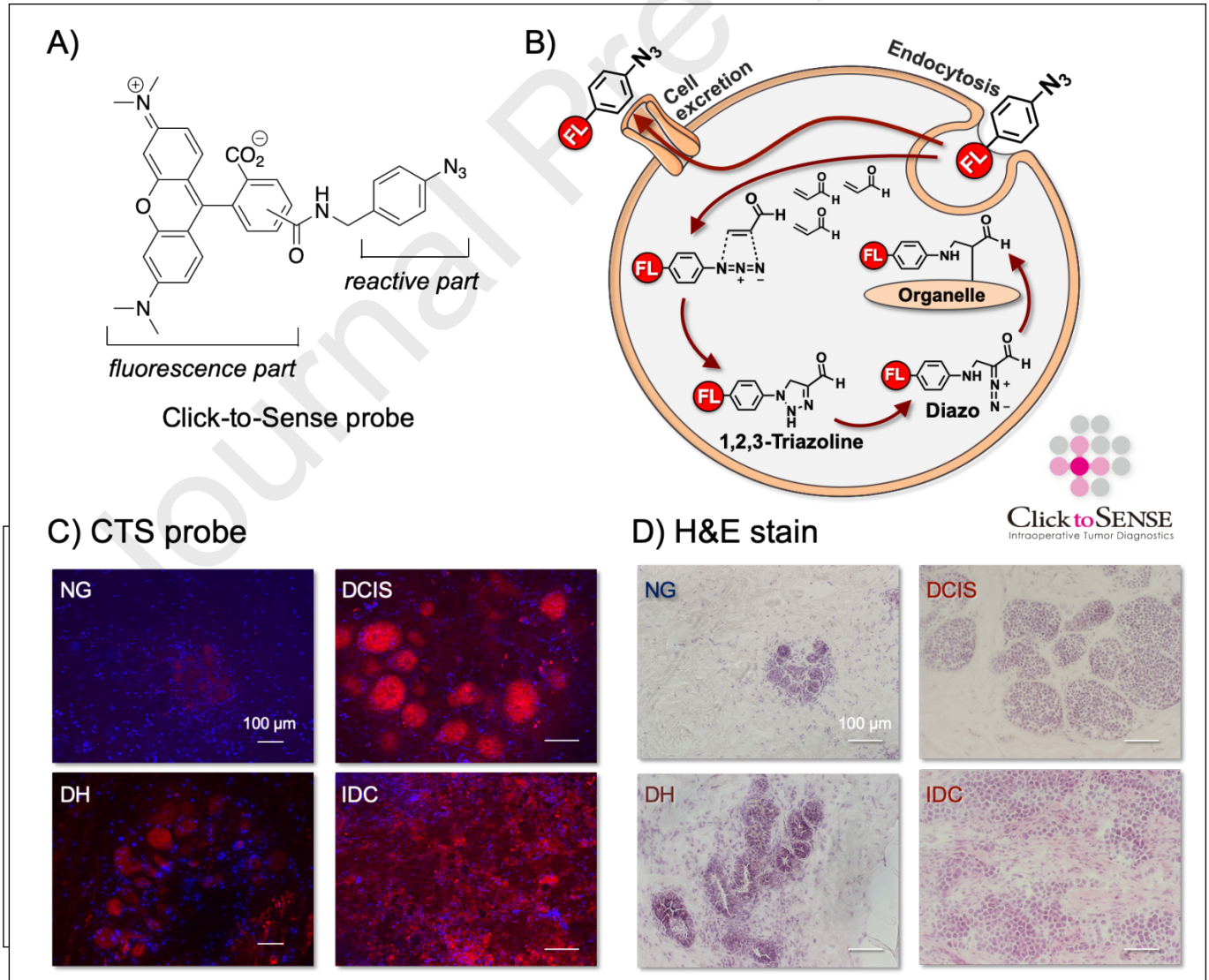

Figure 6. A) Structure of Click-to-Sense (CTS) probe. B) Cascade reaction in cancerous tissue. C) CTS stain and D) H&E stain of tumor tissue from BCS. NG; Normal gland, DH; Ductal hyperplasia, DCIS; Ductal carcinoma in situ, IDC; Invasive ductal carcinoma.

diagnostic criterion of complicated diseases with no other diagnostic methods.

The diagnostic application of acrolein metabolites may have several limitations. As seen in patients with AD or stroke,[52,53,56,57] urinary HPMA is decreased even if the acrolein level is increased, due to GSH depletion. Moreover, the ratio of metabolites changes easily depending on the strength of other metabolic pathways. In addition, FDP-Lys production is considered to be slow[65] and gradually changes to MP-Lys, which is a better substrate of anti-acrolein antibodies.[16] Therefore, the signal from an acrolein-conjugated protein with FDP-Lys would increase over time in a Mab-mediated detection method, although we previously reported an alternative FDP-Lys detection method without Mab. The non-Mab method consists of the reduction of 4-nitrophthalonitrile by FDP-Lys and subsequent fluorescence measurement of the reduced product, 4-amino phthalonitrile (Figure 7).[68] Detected signal from metabolites did not reflect the exact acrolein levels. The FDP-Lys level is in fact different from the extractable acrolein level in patients with AD.[49] From a different perspective, if the patient already has an underlying disease involving oxidative stress and acrolein production, we cannot separately detect the acrolein from other concomitant disease processes. Therefore, the diagnosis is somewhat difficult to interpret based on this data.

A method that allowed the direct application of disease-associated acrolein to the diagnostic and therapeutic substrate at the site of the affected tissue would be extremely advantageous. For example, the pathogenetic diagnosis utilizing a CTS probe is a promising method that could directly reflect acrolein concentration in cells via TIT reaction and could likely minimize the influence of any other underlying diseases. For therapeutic purposes, scavenging acrolein that acts as the main culprit of diseases ameliorated the pathologies in an animal model. This paper presents examples of the potential toxicity caused by acrolein, such as liver injury in AAH[40] and the neurotoxicity of stroke.[56,58] An example of how acrolein toxicity can be mitigated is how the drug Mesna can trap acrolein which causes hemorrhagic cystitis during cyclophosphamide or ifosphamide treatment (Figure 3, arrow 5).[69] On the other hand, the 8-membered ring compound formed with acrolein and polyamine (again categorized as TIT reaction) has been shown to suppress the aggregation of Aβ peptide in AD.[27] The development of more diverse and unique in vivo synthetic chemistry targeting disease-specific acrolein would be useful for the diagnosis and treatment of various diseases.

5. Conclusion

Acrolein or its metabolite levels were significantly increased in the urine, plasma, or tissues of patients with many kinds of diseases, and acrolein is often associated with the initiation or progression of pathological conditions. The influence of acrolein has been suggested in the pathogenesis of many other diseases, especially those related to the nervous system such as spinal cord injury, multiple sclerosis, amyotrophic lateral sclerosis, and Parkinson's disease.[70-73] Oxidative stress conditions and acrolein production are common states in human diseases. Therefore, utilization of acrolein as a substrate based on in vivo synthetic chemistry, especially through TIT reaction, could be a promising therapeutic or diagnostic method that could be applied to a wide range of diseases in the future.

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