Review

Cancer Research

Extracellular DNA: A Bridge to Cancer

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Abstract

DNase I is a secreted enzyme whose function has been presumed to control "waste management" in the human system, by degrading DNA that leaks from dead and dying cells. Emerging studies have instead yielded evidence that DNase I plays a central role in newly defined dynamics of immune and autoimmune diseases, as well as cancer and vascular disorders, including thrombosis. Cancer cells have been reported to be associated with distinctive extracellular structures that facilitate aggregation and implantation. The fact that DNA is a component of such structures and that it plays a role in cancer development is illustrated by direct evidence: DNase I added to tumor cells eliminates the structures and inhibits tumorigenicity of some cancer cell lines. DNase I injected into experimental animals, moreover, results in significant inhibition of metastasis. Despite independent observations of such phenomena in diverse cancers for over 50 years, the potential for using DNase I as a clinical tool to prevent or treat cancer remains unexplored. The discovery of neutrophil extracellular traps has yielded a conceptual framework for interpreting how extracellular DNA may function in cancer development and why it may prove to be an important clinical target in stopping cancer outside the cell. *Cancer Res; 75(20); 4260–4.* ©*2015 AACR.*

Introduction

Since discovery of its role in the propagation of life, deoxyribonucleic acid (DNA) in plants and animals has been considered to function inside the cell, and its presence outside cells was presumed to comprise debris from dead cells (1–3). In 2004 (4), a paradigm shift was set in motion when neutrophil extracellular traps (NET) were described for the first time: In response to defense pathway-inducing signals, extracellular DNA (exDNA) is exported and assembled together with actin, histone, peroxidases, and other reactive oxygen species (ROS) generating proteins into the NET, a sticky matrix surrounding the cell. Pathogens are chemotactically attracted to NETs where they are immobilized and can be killed by antimicrobial components of the trap, including DNA itself (5, 6). Production of extracellular DNase (exDNase) by bacteria, fungi, and other pathogens breaks down the DNA framework and thereby facilitates release from trapping and allows systemic dispersal (1, 4, 5). Acquisition of exDNase, in fact, recently was found to be correlated with the first step in evolution of today's virulent Group A Streptococcus (GAS) strains (7).

A parallel extracellular trapping process operates in plant root tips, whose cells export a complex slimy matrix that specifically attracts, immobilizes, and prevents invasion by pathogens, including bacteria, fungi, and nematodes (3). Newly synthesized exDNA together with >100 proteins, including histone, actin, peroxidases, superoxide dismutase, and other components of ROS pathways, comprises the trap, and treatment with DNase 1 or proteinase K results in 100% loss of resistance to infection (8, 9). Adding antibody to a single 14-3-3 protein within the trap during the infection process, in fact, results in a 50% reduction in resistance (9). Proteins within mammalian NETs also play critical structural, functional, and regulatory roles whose interactions with exDNA in defense processes are just beginning to be described (5, 10–13). These new insights into the role of the extracellular matrix in immune system function may yield novel targets for infectious disease control in agriculture and medicine.

On the other side of the "double-edged" sword of these important findings is the recognition that overproduction or insufficient clearance of DNA, chromatin, and other NET components may be as dangerous to the host as failure to produce traps to control pathogens (14–20). Systemic lupus erythematosis (SLE) and cystic fibrosis, for example, are autoimmune diseases that occur in association with an overabundance of exDNA (1, 18). The hypothesis that NET exDNA overproduction is a contributing factor to signs and symptoms is supported by observations that SLE is associated with reduced DNase I levels that in some cases is associated with altered expression of DNase I encoding human genes (11, 21, 22). In cystic fibrosis patients, treatment with DNase I to reduce exDNA levels relieves respiratory symptoms (23).

Increasing knowledge of NET function has yielded insights into how inflammation that stimulates NET production also may stimulate cancer development: As within the nucleus where genes controlling life processes are assembled through the dynamic properties of the DNA double helix, a DNA-based matrix outside the cell assembles factors needed to trigger the adherence, growth, and metastasis of cancer cells as well as cancer-associated thrombosis (14, 16, 17, 19, 20, 24). As Demers and Wagner (16) expressed it, the extracellular traps provide a "seeding soil" for cancer cells. This concept provides a new basis for interpreting long-standing observations that increased exDNA (also called circulating free DNA or cfDNA), and reduced DNase levels in human blood occur in correlation with cancer development and

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progression (25–33). Studies by independent laboratories around the world, moreover, repeatedly have documented that DNase 1 added to diverse cancer cells inhibits their metastatic potential (30, 34–41).

Increased exDNA Levels in Cancer Patients: Mechanisms?

In 1977, Leon and colleagues (25) found not only that exDNA levels were high in cancer patients, but that a significant reduction in exDNA levels after radiotherapy was correlated with improved clinical condition, whereas failure of exDNA levels to decrease after treatment was correlated with poor prognosis. Since that discovery, increased exDNA levels in human blood repeatedly have been found to be a hallmark of metastatic cancer (41–47). Suggested mechanisms have included DNA release from actively dividing tumor cells (42), tumor lysis, apoptosis, and necrosis (28, 32), but whether these phenomena underlie changes in exDNA levels in cancer patients has remained unclear (41–46). Based on the logical presumption that such "circulating DNA" in cancer patients is derived from dead tumor cells (2, 43), interest in the potential for using the exDNA sequences as a "liquid biopsy" for diagnostic purposes has been high and the phenomenon has been documented in hundreds of papers (32, 48–50). A high degree of variability in the detection of tumor-specific markers in exDNA sequences, however, raises questions about the reliability of exDNA for diagnostic purposes based on the presumption that it is derived from dead tumor cells (32, 33).

Identification of tumor-associated neutrophils (TAN) offers new interpretations for the origin of circulating exDNA levels that in part may explain such results, and defining the mechanism underlying changes in dynamics may yield direct clinical applications (4, 5, 11, 51). Meanwhile, the values for exDNA in blood have been proposed to comprise important clinical information regardless of whether future studies reveal that the exDNA includes tumor sequences and/or is derived of extracellular trap DNA (33). Either model could account for observations that the exDNA of cancer patients can facilitate oncogenic transformation, and that DNase I treatment of cancer cells or the supernatants of cancer patients removes the capacity to metastasize (30, 36, 40, 41). These data highlight potential avenues to control cancer by establishing protocols to define and maintain healthy exDNA and DNase I levels (14).

Decreased exDNase Levels in Cancer Patients

In 1950, Wroblewski and Bodansky (52) first reported reduced DNase ($P < 0.01$) levels in blood of cancer patients compared with healthy controls and individuals with other conditions. Subsequent studies revealed a correlation between DNase levels in blood and treatment response: Thus, DNase I levels in cancer patients increase during remission and after successful interventions, and decrease during metastasis and progression; failure of DNase levels to increase in response to treatment was found to be correlated with poor prognosis (26, 27).

Increased exDNA and Reduced exDNase in Cancer Patients versus Healthy Controls

Most publications have reported results of either exDNA or exDNase levels, but several studies have measured both within the same human subjects (28, 29, 31). Despite the use of small diverse populations, different measurement protocols, and distinct cancers at different stages, highly significant differences ($P < 0.01$–0.0001) were detected: Mean DNase I levels were found to be lower in cancer patients than in healthy controls, and mean exDNA levels were found to be higher in cancer patients than in healthy controls. The basis for the differences has largely been unknown, but empirical studies of the effect of DNase I injections on cancer in animal models, summarized below, have yielded implications of a cause-and-effect relationship.

DNase Effects on Cancer

DNase I injection in mouse using diverse cancer models has been shown to inhibit metastasis and increase survival, without causing negative effects in the test subjects (Table 1). In response to early enzyme surveys suggesting that nucleases inhibit cell growth in tissue culture, de Lamirande (35) carried out direct tests to measure the effect of DNase and RNase on cancer in mice using Ehrlich ascites carcinoma as a model system. Daily injection of DNase starting at 4 days after tumor cell transplantation resulted in a 34% increase in survival time (>95% significance level), and when started at 1 day after transplantation, the survival time was increased by 67% (>99% significance level). RNase injections had no effect on survival. The mechanism was presumed to involve uptake of the enzyme into cancer cells followed by necrosis due to degradation of nuclear DNA.

Table 1. Effect of DNase I injection on cancer in animal models

Cancer	DNase treatment	Effect on metastasis, mitosis, survival	Reference
Ehrlich ascites	i.p. injection ×17, from day 1	67% increase in survival time ($P > 98\%$)	35
	i.p. injection ×19, from day 4	34% increase in survival time ($P > 99\%$)	
		Reduced number of cancer cells	
Lymphatic leukemia	s.c. injection × 29	Reduced dimensions of peripheral lymph nodes	36
		Increased survival (16 weeks vs. 4 weeks)	
		Mitotic index 6× higher in controls than DNase-treated	
Liver metastasis	i.v. injection × 3	Up to 24-day increase in survival	37
Lewis lung carcinoma	i.m. injection × 12	Up to 45% inhibition of metastasis (<0.05)	38
Lewis lung carcinoma	i.m. injection × 10–12	70%–90% inhibition of lung surface metastasis	39
		Reduced levels of plasma exDNA	
		Increased levels of plasma DNase	
Pancreatic cancer	i.p. injection × 7	Reduced migration, adhesion	40
		60% reduction in metastasis at 5 weeks	

Abbreviation: i.m., intramuscular.

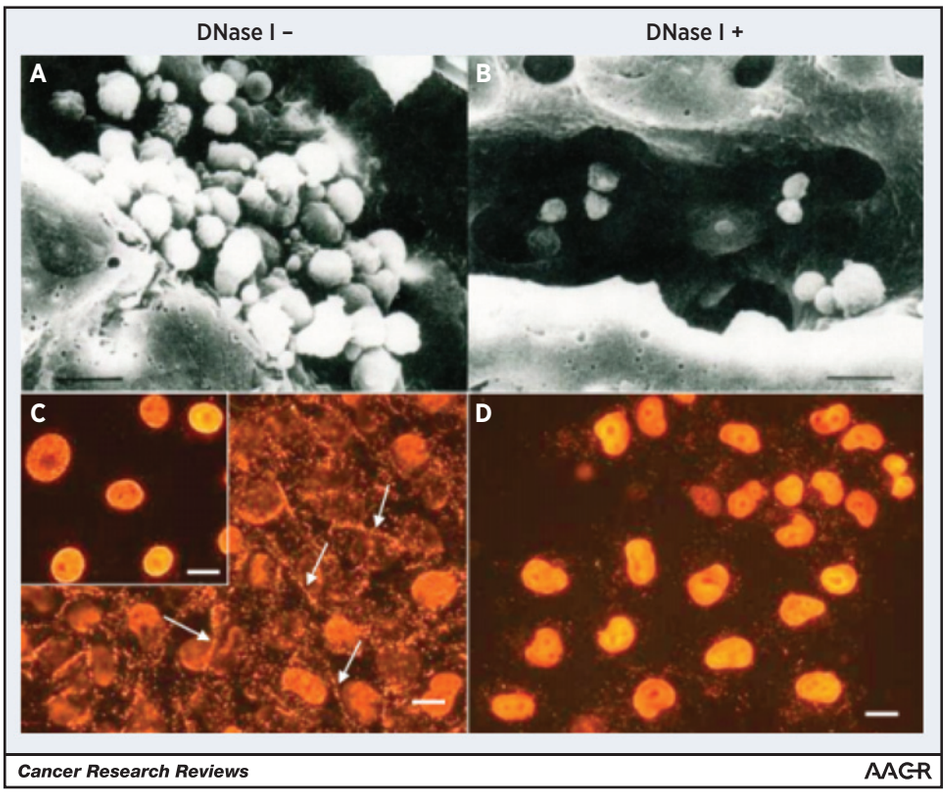

Figure 1.
Effects of DNase I treatment on murine liver tumor cells and human pancreatic cancer cells. A and B, scanning electron micrographs of the liver of mice on day 10 after subcutaneous inoculation with L517Y-ML cells. A, PBS-treated control. Many tumor cells arrested on endothelial cells. B, DNase I–treated case (0. 1 U per mouse). The number of tumor cells is significantly smaller than the control. Endothelial cells are intact. Scale bar, 10 μm. Reproduced with permission from the publisher British Journal of Cancer (Sugihara et al.; ref. 37). C and D, exDNA (arrows) associated with pancreatic cancer cells (left), but not with normal pancreatic cell lines (left, inset). C and D, immunofluorescent stain using DNA antibody was conducted on immortalized normal human pancreatic ductal epithelial (inset) and MiaPacCa-2 pancreatic cancer cells in the absence of DNase I treatment (C) and in the presence of added DNase I (D). Scale bar, 20 μm. Reproduced from Wen et al. (40).

Salganik and colleagues (36) used spontaneous lymphatic leukemia in mouse as a model to document that DNase injections, but not RNase injections, resulted in a measurable decrease in lymph node size compared with controls within a week after starting treatment, and a 12-week increase in survival over controls. The authors found that treatment of leukemic tissue with DNase eliminated the ability to transfer leukemia, with failure to produce a single tumor in response to transplantation. The mechanism was unknown.

Sugihara and colleagues (37) documented that injection of DNase I for 3 days after implantation of tumor cells in a mouse model resulted in increased survival of up to 24 days compared with controls treated with saline injection only ($P < 0.01$). *In situ* electron microscopy revealed that aggregates that developed on the liver after tumor cell transplantation (Fig. 1A) dispersed in subjects treated with DNase I (Fig. 1B). Similar results occurred using a Lewis lung carcinoma model, with significant reduction in metastasis and significant increases in survival in response to DNase I injection (38, 39). The DNase treatments also were correlated with a reduction in plasma exDNA levels and an increase in plasma DNase levels (Table 1).

Based on the observation that cancer cells were found to be associated with mesh-like extracellular structures, which were not observed after DNase I treatment, Sugihara and colleagues (37) speculated that DNA might be part of an extracellular matrix that facilitates implantation. This hypothesis is supported by studies documenting that treatment of diverse cancer cells or their supernatants with DNase I results in loss of the ability to transmit cancer (30, 34, 36, 41). Pancreatic cancer cell lines and DNA antibody were used to provide direct evidence that exDNA strands are indeed present on cancer cells (Fig. 1C), and that they are susceptible to dispersal by treatment with DNase I (Fig. 1D; ref. 40). DNase I treatment of pancreatic cancer cells resulted in reduced migration, adhesion, and invasion, as well as production of the inflammatory chemokine CXCL8 (Table 1). As in previous studies with other cancers, DNase I injections after pancreatic tumor transplantation in mouse resulted in a significant reduction in metastasis.

Clinical Applications of DNase I

A synthesis of over 50 years of independent studies measuring exDNA–exDNase dynamics strongly supports the suggestion by several authors (14, 30, 31, 37) that DNase I application in cancer treatment alone or in combination with other methods is a logical clinical approach. This is especially true now that there is a context for understanding how exDNA arises in response to inflammation and how it may facilitate metastasis unless controlled by adequate DNase I activity. DNase I is a normal component of human blood, and apart from potential antigenic responses to the added protein from nonhuman sources, there is no basis to predict major concerns about clinical dangers. A recombinant human DNase I developed by Genentech has been used for decades to treat cystic fibrosis and empyema in children and adults without reports of significant negative effects on health, and DNase I injections in lupus nephritis patients revealed no significant adverse events (23). Clinical trials using DNase I to treat conditions, including dry eyes and peritonitis, as well as symptoms of head and neck cancer treatment, are under way (https://clinicaltrials.gov).

Successful clinical applications will depend on research to establish when, where, and how self-defense transitions into self-damage, and the role played by exDNA and DNase dynamics in relation to neutrophil proteins that also are integral parts of the immune response (10–13, 53, 54). Empirical studies documenting that dispersal of cancer cells in response to DNase treatment (Fig. 1; Table 1) is correlated with inhibition of metastasis are consistent with the hypothesis that aggregation of cells within the tumor microenvironment is a critical turning point for "seeding" of tumors, and therefore may be a logical target for prevention (16, 55). New insights into neutrophil proteins within the tumor microenvironment include observations by Trejo-Bercerril and colleagues (30) that injection of protease together with DNase offsets tumor development in response to injection with HeLa cells. Using a combination of treatments that help prevent assembly of DNA and proteins into a reactive matrix that triggers metastasis is an approach to consider. Plant proteases have been used as adjunct cancer treatments with some reported benefits, but the mechanism was unknown (56).

Concluding Remarks

DNase I was long ago assigned a role as a "waste-management" enzyme based on the presumption that DNA outside the cell is only present as a by-product of dead and dying cells that disintegrate and disperse their contents. Instead, exDNA-based traps and exDNases that modify them are integral components of programmed responses of the eucaryotic immune system. Empirical studies documenting effects of DNase on cancer (Table 1; Fig. 1) are consistent with a model in which chronic or excessive inflammation triggers increased exDNA production within a context of insufficient exDNase activity to regulate its effects on the host. As such, there is a critical need to explore the potential to develop clinical strategies to hit the exDNA target that is present on cancer cells but not healthy cells, to stop disease outside the cell, using tools that will not inflict the level of injury on patients that most current cancer treatments cause. Multidisciplinary research will be needed to define activities, diet, diurnal rhythms, age, gender, medications, and other factors that may act as inducers and suppressors to facilitate DNase production and/or access of the enzyme to key components of the exDNA matrix (57, 58). In the meantime, studies to establish standardized protocols to define a healthy range for serum and/or plasma DNase levels in humans and to explore the impact on cancer and other diseases when levels are restored to that range are long overdue. Tools to detect NET exDNA sequence signature(s) that define its function and delineate it from nucleic acids extruded nonspecifically from disintegrating cells will be especially critical.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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