REVIEW

Niacin and Carcinogenesis

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Abstract: The dietary status of niacin (vitamin B3) has the potential to influence DNA repair, genomic stability, and the immune system, eventually having an impact on cancer risk, as well as the side effects of chemotherapy in the cancer patient. In addition to its well-known redox functions in energy metabolism, niacin, in the form of NAD, participates in a wide variety of ADP-ribosylation reactions. Poly(ADP-ribose) is a negatively charged polymer synthesized, predominantly on nuclear proteins, by at least seven different enzymes. Poly(ADP-ribose) polymerase-1 (PARP-1) is responsible for the majority of polymer synthesis and plays important roles in DNA damage responses, including repair, maintenance of genomic stability, and signaling events for stress responses such as apoptosis. NAD is also used in the synthesis of mono(ADP-ribose), often on G proteins, with poorly understood roles in signal transduction. Last, NAD and NADP are required for the synthesis of cyclic ADP-ribose and nicotinic acid adenine dinucleotide (NAADP), two mediators of intracellular calcium signaling pathways. Disruption of any of these processes has the potential to impair genomic stability and deregulate cell division, leading to enhanced cancer risk. There are various sources of evidence that niacin status does have an impact on cancer risk, including animal models of leukemogenesis and skin cancer, as well as epidemiological data from human populations.

Introduction

Niacin deficiency in humans causes the disease pellagra, which is characterized by the four Ds, representing diarrhea, dementia, sun-sensitive dermatitis, and death (1). For many years, these unusual symptoms were hard to understand in the context of redox functions of NAD(P) cofactors. Insight into these pathologies has improved with the discovery of various other reactions which consume NAD, including the formation of poly ADP-ribose (pADPr), mono-ADP-ribose, cyclic ADP-ribose, and nicotinic acid adenine dinucleotide (NAADP) (Fig. 1). Although clinical niacin deficiency is no longer common in developed countries, subclinical deficiencies appear to be of concern (2,3), and certain populations, such as cancer patients, appear to be at risk for niacin deficiency (4,5). This area requires further work, but a limited data set showed that all cancer patients not taking intravenous vitamin supplements were niacin deficient, and rather large niacin supplements appeared to be required to normalize N-methyl-nicotinamide excretion (4).

Niacin Status and Human Cancer Incidence

There is a shortage of knowledge on the impact of niacin on cancer risk in human populations. It is known that cancer patients tend to be deficient in niacin at a time when they are exposed to large doses of genotoxic drugs during chemotherapy (4,5). Between 5% and 10% of surviving chemotherapy patients develop secondary cancers, especially leukemias (6). Although animal models suggest that niacin deficiency enhances this risk (7), there are no human data available to further define this risk.

Although developed countries generally supplement niacin in cereal products, there may still be a significant proportion of these populations experiencing subclinical niacin deficiency (2,3,8). Niacin and riboflavin were supplemented in one of the treatment arms of the Linxian trials in China (9). Although these supplements did not provide any benefit to the oral/esophageal cancers in this study, there are various ways to interpret these results. The duration of the study was probably too short to examine the role of niacin during cancer initiation. In addition, the high esophageal cancer incidence in this population is associated with heavy contamination by fumonisin mycotoxins (10), which appear to promote carcinogenesis by a rather unique mechanism that may not be responsive to niacin status (11).
Figure 1. A general scheme of NAD metabolism, showing dietary sources of niacin, redox reactions, and ADP-ribosylation reactions.

Human patients with hypercholesterolemia often are treated with high doses of nicotinic acid (greater than or equal to 3 g/day). Studies on the impact of these treatments on cancer incidence are few in number, cover relatively short periods of time, and represent late stages in the carcinogenic process. However, nicotinic acid therapy did not seem to cause the small increase in cancer incidence observed in populations using a variety of other nonstatin drugs to lower blood cholesterol (12). Interestingly, nicotinic acid use for 6 years by patients with cardiovascular disease led to a decrease in all-cause mortality measured 8 years after the drug use was discontinued (13).

Epidemiological studies about niacin status and human cancer incidence often find significant associations, but interpretation of these is also difficult. In a variety of countries, including Iran, Africa, Italy, Switzerland, and the United States, maize (corn) consumption (which causes niacin deficiency), or low levels of estimated niacin intake, have been associated with an increased frequency of gastric, colon, oral, or esophageal cancers (14–19). This is interesting from the perspective that niacin deficiency may promote cancer at this site. However, these experiments suffer from covariance between maize consumption and fumonisin exposure, and/or niacin status and fruit and vegetable consumption. No work has been done on the epidemiology of niacin deficiency and skin cancer, although animal models of niacin deficiency (21) and human familial DNA repair defects that mimic the sun sensitivity of pellagra (22) are associated with an increase in skin cancer risk.

Niacin Status and Genomic Instability and Carcinogenesis in Animal Models

Bryan reviewed this topic in 1986 and noted that all of the work to that point had examined pharmacological intakes of nicotinic acid and nicotinamide, rather than niacin deficiency (23). Toth fed diets to mice containing 10 g nicotinamide/kg (>300-fold above requirement) and noted no effects on spontaneous cancer incidence (24). In combination with exposure to genotoxic agents, high levels of nicotinamide protected against bracken fern and urethane-induced cancers, while enhancing nitrosamine and heliotrine-induced cancers (23). Multiple mechanisms appear to be influencing the response to pharmacological intakes of niacin.

Our work on the characterization of niacin deficiency in whole animal models has created a complex picture. Many tissues, such as the liver and lung, have very high NAD levels, are resistant to NAD depletion during dietary niacin deficiency, and are not sensitized to DNA damage while niacin deficient (25,26). In contrast, certain tissues, such as bone marrow and skin, are very sensitive to niacin deficiency. We demonstrated that niacin deficiency in rats increased the severity of acute ethyl nitrosourea (ENU)-induced anemia and leukopenia (bone marrow suppression) (27). During niacin deficiency, ENU-induced anemia was associated with a decreased ability of the marrow to produce new red blood cells. In longer term studies, niacin deficiency caused a decreased latency and increased number of ENU-induced cancers, most of which were leukemias (7). Niacin deficiency also caused an 80% decrease in bone marrow NAD and an almost complete loss of poly(ADP-ribose) (pADPr) pools, while ablat-
cind deficiency caused a significant delay in repair from 12 to 30 h after the initiation of DNA damage (unpublished data). Sister chromatid exchanges and micronuclei are increased three- and sixfold, respectively, by niacin deficiency alone (29), and niacin deficiency increases the induction of micronuclei by ENU (unpublished data).

### Skin Cancer and Immune Function

As suggested by the sun sensitivity of pellagra, models of UV exposure of rodents have shown that the skin is sensitized by niacin deficiency (21). In findings that could be very important to human health, skin appears to be further protected from UV irradiation by pharmacological supplementation with niacin (30). Mild niacin deficiency in mice has been shown to significantly decrease skin NAD levels and increase the development of UV-induced skin tumors (21). Conversely, pharmacological intakes of niacin, up to 10 g/kg diet, decreased UVB-induced skin cancer incidence to 28% of that seen in niacin-adequate mice (30). In these experiments, skin NAD levels increased with dietary niacin, indicating a possible role in DNA repair and genomic stability in skin cells. However, these experiments also showed that the immune system, which is known to be suppressed by UV exposure, was more active toward skin tumors as niacin supplementation was increased. This effect on the immune system could be caused by the synthesis of cADPr or NAADP by CD38 protein in immune cell lineages (31).

### PARP and Genomic Stability

Sun-sensitive dermatitis is suggestive of problems with DNA repair, similar to familial syndromes of sun sensitivity linked to mutations in DNA repair genes (32). The connection between niacin and sun sensitivity was illuminated by the discovery that NAD is required for the synthesis of pADPr by the enzyme poly(ADP-ribose) polymerase-1 (PARP-1) (Fig. 2). PARP-1 is a zinc finger protein that binds specifically to strand breaks in DNA. This binding causes catalytic activation, leading to the synthesis of pADPr on a variety of nuclear proteins, the most predominant of which is PARP-1, itself, termed “automodification” (33). pADPr is highly negatively charged, and modified proteins will tend to lose affinity for DNA, which is also anionic. Automodified PARP-1 will dissociate from DNA strand breaks, because of charge repulsion, allowing repair to proceed (34).

Recent reports that PARP-null mice still synthesize small amounts of pADPr (35) have been validated by the discovery of six other pADPr-synthesizing enzymes, including two other nuclear enzymes (PARP-2, PARP-3) (36,37), a vault-associated protein (VPARP) (38), and two telomere-associated proteins (tankyrase-1 and 2) (39). Analysis of genome sequence data suggests that the number of PARP enzymes may expand to 16 or more (40). PARP-2 has been found to be similar to PARP-1 in function; both are catalytically activated by DNA damage, they heterodimerize, and both interact with other DNA repair proteins, such as XRCC1 (41). Disruption of both PARP-1 and 2 genes causes embryonic lethality (42).

Tankyrase-1 binds TRF1, which is a negative regulator of telomerase. Tankyrase-1 is capable of synthesizing pADPr on TRF1, forcing it to be released from its binding at the telomere, allowing telomerase to access and elongate the end of the chromosome (43). Tankyrase-2 shares 85% amino acid identity with tankyrase-1, has a similar subcellular distribution, and also interacts with TRF1 (44). In vitro, tankyrase-1 is responsive to NAD concentrations through the physiological range, producing larger sized pADPr as the NAD concentration is increased (39). This is similar to the behavior of PARP-1 and suggests that tankyrase also will be influenced by dietary niacin status.

The function of VPARP, and vault particles in general, is poorly understood. Although VPARP is found mainly in the cytosol, associated with vault particles, PARP-1, tankyrase-1, and VPARP also have been found to localize to centromeres or spindle apparatus during mitosis (38,45,46), and there may be a coordinated role for pADPr synthesis during the segregation of chromosomes.

There are a variety of ways that PARP activities may be involved in the maintenance of genomic stability, and these are discussed below.

### PARP and Excision Repair

There are two excision repair pathways, base excision repair (BER) and nucleotide excision repair (NER) (47). BER is responsible for the repair of small alkylations and oxidant damage, which do not cause a large deformation of chromatin structure. NER is important for bulky adducts and photo products caused by UV light. BER is thought to be the more critical pathway, because there are no human gene defects to essential proteins in this pathway, whereas Xeroderma pigmentosum is an example of a sun-sensitivity disease caused by various defects in the NER pathway. BER may be more essential because of the constant formation of oxidant DNA lesions and small alkylations, or the requirement for repairing large numbers of spontaneous depurinations.

Results on the role of PARP-1 in excision repair have been conflicting. It generally is believed that PARP-1 is involved in BER, and not in NER. Numerous studies have shown that competitive inhibition of PARP delays strand break rejoining and increases the cytotoxicity of many carcinogens (48). Two studies have shown similar results using niacin-deficient cell culture medium (48,49). Similarly, expression of catalytically inactive PARP-1 DNA-binding domains inhibit excision repair (50). This developing dogma was challenged by data from simplified in vitro repair systems, which showed that NAD was required for repair when PARP was included, but repair proceeded at an optimal rate when both PARP and NAD were removed (34). The results from different PARP-1–null mouse models have differed for the role of PARP-1 in BER (51,52). This controversy has been clarified somewhat by the finding that
PARP-2, a 62-kDa nuclear protein, also synthesizes pADPr in response to DNA damage and is involved in BER (41). Deletion of both PARP-1 and 2 causes embryonic lethality (42), consistent with the idea that these two proteins share some redundancy in an essential role in BER.

It is important to understand the differences between these models, and eventually how they reflect on the impact of niacin deficiency in the whole animal environment. Competitive inhibitors of PARP-1, niacin depletion of cultured cells (48,49), and niacin-deficient rat bone marrow cells (7) all harbor catalytically inactive PARP-1 molecules, which presumably bind persistently to DNA strand breaks because of a lack of automodification. PARP has a relatively high Km for NAD\(^+\) (1), and a loss of PARP activity

Figure 2. The synthesis of poly(ADP-ribose) on protein acceptors and its degradation by poly(ADP-ribose) glycohydrolase.
appears to occur at stages of NAD depletion (e.g., 50% of control NAD\(^+\) in cultured cells) (49), which do not affect basic redox reactions and energy metabolism, as judged by cell division (48).

A blockage of repair by PARP-1 under low NAD\(^+\) conditions is in agreement with Satoh’s simplified in vitro model (34). This is also the case with expression of PARP-1 DNA-binding domains, which occupy strand breaks, preventing access by PARP-1 or other repair enzymes. Although there have been conflicting results from PARP-1-null models, it appears that excision repair can proceed (at various rates) in the absence of PARP-1, but not in the presence of catalytically inactive PARP. If niacin deficiency is able to impair both PARP-1 and 2 activity, this also may have a severe impact on cell function. Despite the fact that excision repair pathways may be somewhat intact in PARP-1–null cells, all of the PARP-1–null mouse models have displayed drastic sensitivity to DNA damage, including acute radiation and nitrosourea sensitivity (53) and increased nitrosamine-induced carcinogenesis (54). This suggests that PARP-1 has important roles in the maintenance of genomic stability, including the possibility that excision repair may proceed at a normal rate, but lack accuracy.

**PARP and Recombination**

The rapid synthesis of negatively charged pADPr at DNA strand breaks may repel other free ends of DNA, thereby inhibiting homologous and nonhomologous recombination events. An increase in sister chromatid exchange (SCE) frequency (homologous recombination) is a universal observation among the various models for PARP-1 disruption, including competitive inhibition (55), DNA-binding domain expression (56), and PARP-1–null mouse models (57). SCE frequencies also were elevated (fourfold) in PARP-2–null mice (42), showing an overlap in function for these two proteins in both repair and control of recombination. SCE frequencies do not represent DNA damage per se but are thought to be an indicator of increased susceptibility to nonhomologous recombination events that lead to the chromosomal translocations responsible for many cancers, especially leukemias. Telomeres are also a site of translocation events that contribute to carcinogenesis and tankyrase-1 and 2 and may play a role in inhibition of telomeric translocations. Tankyrase activity could accomplish this either by maintaining the length of the telomere, or by electrostatic repulsion of other DNA strands.

**PARP and Regulation of p53**

p53 is a protein that plays a central role in cellular processes such as cell cycle arrest and apoptosis in response to DNA damage (58). After genotoxic stress, p53 protein is stabilized and activated by several different posttranslational modifications, including phosphorylation, acetylation, and poly(ADP-ribosyl)ation (59,60). p53 binds to DNA and mediates transactivation of target genes involved in the execution of cell cycle arrest (e.g., p21\(^{WAF1}\), GADD45, and B99) and apoptosis (e.g., Bax, Fas/APO1, Killer/DR5) (58). It has been shown that PARP inhibition results in decreased basal p53 protein levels and impairs p53 stabilization after DNA damage (61–65). The functionality of p53 in PARP inhibited systems has been debated, with some researchers finding no effect of PARP inhibition on the transcriptional activation of p53 responsive targets (63,66), whereas others have found that transcriptional activation of p53-responsive genes such as p21\(^{WAF1}\) and mdm-2 are impaired (62,64,67). The biological relevance of impaired p53 function has been evaluated for the ability of cells to undergo apoptosis and/or cell cycle arrest. Again, the literature is divergent with some investigators reporting impaired apoptosis (61) and cell cycle arrest (68,69), whereas others conclude that p53 activation and function are PARP independent (63).

Evidence to support the proposed interrelationship between PARP and p53 stems from the discovery of pADPr-binding motifs in the p53 DNA-binding domain and oligomerization domain that enable strong noncovalent interaction between free pADPr and pADPr-bound PARP (70). In addition, earlier studies have shown that p53 is posttranslationally modified with pADPr (covalent interaction) in vitro (71,72) as well as during the early stages of apoptosis in vivo (60,73). It has been proposed that this type of modification of p53 with covalent and noncovalent associations with pADPr regulates the DNA-binding properties of p53 (64,70,74). Protein–protein interactions between p53 and PARP have also been documented in several studies in which p53-PARP coimmunoprecipitate with either anti-p53 or anti-PARP antibodies (67,71,72).

**PARP and Telomere Regulation/Stability**

Telomere shortening causes senescence and telomerase activity increases during neoplastic transformation (75). Telomeres also anchor chromosomes to the nuclear membrane and cytoskeleton and appear to play a role in prevention of chromosomal degradation and recombination (76). Reports from the first PARP-null mouse indicated that a lack of PARP expression caused telomere shortening and chromosomal instability, with an increase in end to end fusions (77). This suggested that PARP was important in the regulation of telomere length and control of recombination events at the telomere. Reports from the second PARP-null model also found chromosomal instability, but no change in telomere length or end capping (78). The difference between these two models may be related to the genetic background, specifically the expression of tankyrase-1 and 2, newly discovered pADPr-synthesizing enzymes that function at the telomere (further discussion below).

**PARP and Apoptosis**

PARP-1 has long been associated with caspase-dependent apoptosis, as an early marker for specific proteolytic degradation. Some experiments have shown that this cleavage of
PARP-1 plays a direct role in the apoptotic process (79). It is now also apparent that PARP-1 also participates in an important caspase-independent pathway. A protein referred to as apoptosis-inducing factor (AIF) causes caspase-independent apoptosis when it is relocated from the mitochondria to the nucleus. This translocation is dependent on the catalytic activity of PARP-1, because it is prevented by PARP inhibitors or disruption of the PARP-1 gene (80). If apoptosis is defective, cells with extensive DNA damage may survive and go on to generate neoplasms. We have observed a decrease in etoposide-induced apoptosis in bone marrow cells of niacin-deficient rats, and an increase in pharmacologically supplemented rats, relative to pair-fed controls (unpublished data). This correlates with our observed levels of pADPr (7,28) and is consistent with the idea that dietary niacin status could be influencing apoptotic efficiency through the translocation of AIF. Given that niacin deficiency appears to delay excision repair, and prevent apoptosis, the potential exists for an increased fraction of damaged cells evading apoptosis in the niacin-deficient state.

PARP and Mitotic Spindles

The observation that cells of PARP-1–null mice exhibit frequent loss or gain of chromosomes (81) suggests problems in mitotic spindle function during chromosomal segregation. In support of this finding, PARP has been found to localize to centromeres during cell division (45) and shows high-affinity binding to centromeric DNA sequences (82). Interestingly, two other pADPr-synthesizing enzymes (VPARP and tankyrase-1) show partial localization to the mitotic spindle or centrosome during mitosis (38,46). In addition, PARP-2–null mice display chromosome missegregation and kinetochore defects (42). A decrease in catalytic function of any of these alternate PARP enzymes may contribute to problems in chromosome sorting during niacin deficiency.

Mono-ADP-Ribosylation and Cyclic ADP-Ribose Formation

There are many mono-ADP-ribosyltransferases in most cells, with poorly defined roles. Many of these posttranslational modifications occur on G proteins and may play a role in regulating signal transduction associated with cell division (83). Mono-ADP-ribosylation also is involved in regulating cytoskeletal elements and the stress response protein, GRP78 (83), both of which could play a role in genomic stability. Niacin also is required for the synthesis of cyclic ADP-ribose and the newly discovered molecule NAADP, both of which are involved in the regulation of intracellular calcium signaling pathways (84). Niacin deficiency is likely to have some impact in these areas of metabolism that could be related to cell cycle and carcinogenesis. For example, mice with disrupted CD38 (a major ADP-ribose cyclase) display changes in apoptosis in bone marrow hematopoietic lineages (31). If niacin deficiency caused a similar impairment of cyclic ADP-ribose formation, it could predispose to leukemogenesis during exposure to bone marrow-damaging agents.

Non–ADP-Ribosylation Roles for Niacin in Genomic Stability

NADPH is synthesized in the pentose phosphate pathway and is the engine that drives detoxification of oxygen radicals by glutathione peroxidase. This enzyme converts hydrogen peroxide to water, preventing the formation of highly damaging hydroxyl radicals, which can react with all cellular macromolecules, including DNA. The formation of mutagenic DNA lesions, such as 8-hydroxy-deoxy-guanosin, will be increased during niacin deficiency if oxidant stress is an important issue.

An additional role for NAD in genomic stability may be through the action of SIR2, an NAD-dependent histone deacetylase (85). Deacetylation leads to a more compact chromatin structure and gene silencing. It also appears to protect sensitive areas of chromatin, such as telomeres, against translocation events and to play a role in extended life span associated with caloric restriction (86). In theory, niacin deficiency could lead to a more open DNA structure, with more active gene expression and greater sensitivity to damage and translocation events.

One type of posttranslational modification of p53 after DNA damage is acetylation, and SIR2 also catalyses NAD-dependent deacetylation of p53 (87). The effects of p53 acetylation are not well understood, but acetylation appears to enhance p53 stability and accumulation by inhibiting ubiquitination (59). Acetylation also may enhance transcriptional activation by p53, although this is controversial. In theory, niacin deficiency could cause an accumulation of active p53. Experimentally, we have observed overexpression of larger molecular weight forms of p53 in rat bone marrow during niacin deficiency, which could be consistent with accumulation of acetylated forms, but p53-dependent functions such as cell cycle arrest and apoptosis are impaired (unpublished data). The effect of niacin deficiency on p53 function requires further work.

Metabolic Basis for the Effects of Niacin Deficiency In Vivo

PARP-null mice reflect the role of pADPr formation by PARP alone in a whole animal setting, but niacin deficiency creates an environment that contains PARP protein that may be catalytically limited or inactive (7). This is similar to competitive inhibition of PARP, or expression of PARP DNA-binding domains, conditions known to inhibit excision repair and create genomic instability. However, niacin deficiency also has the potential to disrupt at least six other pADPr-synthesizing enzymes, a multitude of mono-ADP-ribosyltransferases, formation of cyclic ADP-ribose and host of redox reactions. Thus, it is important to view niacin deficiency as a unique com-
Dietary Niacin

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\text{Redox reactions} \quad \rightarrow \ ?
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\text{Cyclic ADP-ribose} \quad \text{NAADP} \quad \text{Mono ADP-ribose} \quad \rightarrow \ ?
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\text{NAD/NADP}
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\text{Telomere stability}
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\text{DNA damage} \quad \rightarrow \ ?
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\text{pADPr synthesis}
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\text{p53 expression} \quad \rightarrow \ ?
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\text{Cell cycle arrest} \quad \rightarrow \ \text{AIF} \quad \rightarrow \ \text{PARP-1} \quad \text{PARP-2} \quad \text{DNA repair}
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\text{Apoptosis} \quad \rightarrow \ ?
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\text{Chromosome sorting}
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Figure 3. Processes involved in genomic stability and potential sites of interaction with niacin status and poly(ADP-ribose) (pADPr) metabolism. Apoptosis inducing factor (AIF) is an important mediator of pADPr-dependent apoptosis. PARP-1 and 2 are clearly involved in excision repair of DNA damage. pADPr is involved in the regulation of p53 expression, which controls cell cycle arrest and apoptosis. Tankyrase-1 and 2 (TANK) are involved in telomere lengthening and stability. Proper sorting of chromosomes may involve PARP-1 and 2, VPARP, and TANK. Signaling pathways controlled by other forms of ADP-riboseylation reactions should be the subject of future work.

Summary

There is a growing body of evidence linking the metabolic roles of niacin with processes involved in carcinogenesis, including genomic instability, DNA repair, and regulation of cell division and apoptosis. Animal models show that niacin status influences carcinogenesis in a tissue-specific manner, which reflects the greater tendency for proliferative tissues to become niacin deficient. There is a lack of good data on niacin status and cancer risk in humans, and we hope this will be corrected with future work in this area.

Acknowledgments and Notes

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Submitted 30 September 2002; accepted in final form 2 July 2003.

References

16. Harrison LE, Zhang ZF, Karpeh MS, Sun M, and Kurtz RC: The role of dietary factors in the intestinal and diffuse histologic subtypes of gas-


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