

New Doctorial Cancer Research

The Role of Nonsense-Mediated mRNA Decay (NMD) in the Regulation of *CDH1* Gene Expression and Cancer Development

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E-cadherin is an adhesion molecule that acts as a tumor-suppressor protein by inhibiting tumor-cell invasion and metastasis as a result of its ability to mediate cell-to-cell adhesion. Germline mutations in the E-cadherin gene (*CDH1*) are the underlying genetic defect responsible for hereditary diffuse gastric cancer (HDGC), with or without cleft lip¹ (OMIM 192090). An unusually high percentage (almost 80%) of *CDH1* mutations generates premature termination codons (PTCs). I hypothesized that transcripts from such mutant genes are degraded by nonsense-mediated decay (NMD), an mRNA quality-control mechanism that recognizes and degrades transcripts harboring PTCs. To test this hypothesis, I examined whether PTC-containing *CDH1* transcripts are downregulated by NMD. Using an allele-specific expression (ASE) assay to differentiate between mutated and wild-type *CDH1* alleles, I observed that PTC-containing *CDH1* mRNAs are strongly downregulated in normal gastric tissue from several asymptomatic *CDH1* mutation carriers.² *CDH1* transcripts harboring PTCs in the KATO-III gastric cancer cell line were upregulated in response to protein-synthesis inhibitors (which inhibit NMD by blocking stop codon recognition) or by depletion of the NMD factors UPF1, SMG1, or eIF4AIII, providing definitive evidence that NMD is responsible for *CDH1* transcript downregulation.² Evidence suggests that NMD modulates the phenotype of numerous diseases. In some diseases, NMD function appears to reduce the severity of the phenotype by downregulating the expression of truncated proteins with dominant-negative activity. In other diseases, NMD appears to increase disease severity by degrading transcripts encoding truncated proteins, which retain residual function. I examined the role

of NMD in determining the phenotype of HDGC patients caused by truncating mutations in the *CDHI* gene. Analysis of clinical data from 264 HDGC patients harboring *CDHI* alleles with PTCs at a wide variety of different positions indicated an association of their predicted ability to induce NMD with an earlier age of onset of gastric cancer.² Furthermore, we obtained evidence that haploinsufficiency due to NMD and inactivation of the second *CDHI* allele by promoter methylation is necessary for diffuse gastric cancer development.³ Additionally, we evaluated the use of *CDHI* ASE in a HDGC family under chromoendoscopic surveillance as a potential diagnostic method. *CDHI* ASE was able to detect both downregulation of PTC-containing alleles in asymptomatic carriers, as well as *CDHI* promoter methylation of the wild-type allele in a diffuse gastric tumor. Altogether, this dissertation provides compelling evidence that NMD is detrimental for HDGC patients. The implication of this finding is that it will be important to develop approaches to detect and inhibit NMD as potentially useful diagnostic and therapeutic approaches in gastric tumors.

Comment by Miles Wilkinson, PhD

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Hereditary syndromes that predispose to cancer are responsible for 1% to 10% of all cases of cancer and represent a serious burden for patients. Dr. Karam's dissertation focused on one such syndrome—hereditary diffuse gastric cancer (HDGC), which is caused by germline mutations of the E-cadherin gene (*CDHI*). In his thesis, he provided compelling evidence that HDGC is caused by transcripts downregulated by an important mRNA quality-control mechanism known as nonsense-mediated decay (NMD). Past studies have shown that NMD is a modulator of the phenotype of several diseases, including cystic fibrosis and Duchene muscular dystrophy. Dr. Karam was the first to show that aberrant E-cadherin mRNA is downregulated by NMD in *CDHI* mutation carriers. He definitively demonstrated that NMD is responsible for this downregulation, using several different approaches in a diffuse gastric cancer cell line. Additionally, he uncovered an association of predicted NMD activity with an earlier age of onset of gastric cancer in a series of E-cadherin mutation carriers. This, as well as the observation of lower cumulative risk in patients harboring a *CDHI* mutation that escapes NMD, suggests that NMD has a detrimental effect on the clinical progression of HDGC. His results reinforce the idea that strong downregulation of E-cadherin (and probably other tumor suppressors in other syndromes), both by NMD and by second-hit inactivation of the wild-type allele, is a frequent and key event triggering cancer development. Consequently, NMD is a potentially useful therapeutic target in this syndrome. To analyze the effect of approaches targeting NMD, Dr. Karam investigated by microarray analysis the effect of NMD inhibition in human cancer cell lines. This analysis revealed an intricate connection between mRNA and protein quality-control mechanisms. This has large implications, as it will

probably influence how “NMD inhibition” therapy is used to prevent or treat cancer or both. In summary, I believe that Dr. Karam’s thesis work has opened up novel molecular approaches—involving the NMD RNA surveillance pathway—toward both the diagnosis and prevention of gastric tumors.

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