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DNA methylation as a potential regulator of NOD like receptor expression and pro-inflammatory activity

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Introduction: NOD-like receptors (NLRs), of which NOD1 and NOD2 are the best characterised, are a class of intracellular pathogen recognition receptors involved in innate immune responses and are expressed in monocytic cells and the intestinal epithelium [(1)]. Stimulation of NOD1 and NOD2 receptors with components of bacterial peptidoglycan results in cytokine production [(2)]. Aberrant NLR expression has been associated with chronic inflammatory diseases [(3)]. Mechanisms underlying NLR expression regulation are currently unknown. DNA methylation has been shown to modify gene expression and is effected by DNA methyltransferase enzymes [(4)]. We hypothesised that DNA methylation may play a role in regulating NOD activity.

Method: To analyse the effect of DNA methylation on basal NOD1/2 expression; THP-1 monocytic or HCT116 intestinal epithelial cell lines were treated with a DNA methyltransferase 1 inhibitors; either 5μM 5-Azacytidine (5-Aza) or 500nM 5-Aza-2-deoxycytidine (5-Aza-dC) for 72 hours, after which NOD1/2 expression was analysed at the mRNA and protein level (n≥6) by quantitative polymerase chain reaction (QPCR) and western blotting, respectively. To assess NOD1/2 pro-inflammatory activity 5-Aza or 5-Aza-dC treated cells were subsequently stimulated for 6 hours with NOD1/2 ligands, after which expression of TNF-α and IL-6 were quantified at the mRNA level (n≥6) by QPCR. Data was analysed using independent t-test or two-way ANOVA analysis where appropriate, followed by appropriate post-hoc tests.

Results: Basal expression studies in THP-1 monocytic cells revealed 5-Aza significantly increased both NOD1/2 mRNA (p<0.001) and protein (p<0.05). 5-Aza-dC significantly increased NOD1 protein (p<0.01) as well as NOD2 mRNA and protein (p<0.001). Basal expression studies in HCT116 intestinal epithelial cells revealed 5-Aza did not significantly alter NOD1/NOD2 mRNA (p>0.05) but did increase NOD1/NOD2 protein (p<0.05). 5-Aza-2-dC significantly increased NOD2 mRNA (p<0.01) and NOD1/NOD2 protein (p<0.01). Pro-inflammatory activity studies in THP-1/HCT116WT cells revealed that 5-Aza/5-Aza-dC treatment increased TNF-α and IL-6 mRNA expression in both cell lines in response to NOD1/2 ligands (p<0.001).

Conclusion: These findings suggest that NOD1 and NOD2 receptor expression and pro-inflammatory activity are epigenetically regulated and could potentially act as a novel drug target for chronic inflammatory disorders including inflammatory bowel diseases.

References:

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