

## Commentary & View

# Adhesion- and migration-related side effects of phosphothioated CpG oligodeoxynucleotides

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Nucleic acid oligodeoxynucleotides (ODN) are increasingly used in biological research and in clinics where they are used for both diagnostic and therapeutic purposes. In order to increase the stability and efficacy of ODNs, various chemical modifications have been applied to create nucleic acid derivatives that are not recognized by endogenous nucleic acid cleavage mechanisms. One of the most common and cost-effective modifications is the phosphothioate (PTO) modification. The PTO modification is implemented mainly in antisense ODN, but also in ODN that were shown to activate members of the toll-like receptor (TLR) family such as TLR3 (poly-I:C), TLR8 (ssRNA) and TLR9 (CpG). We recently found that PTO-ODN aimed at activating TLR9 induce a non-TLR9-specific detachment phenotype in a growth-substrate dependent manner. Moreover, we found that unmodified and PTO-modified TLR ligands induce distinct patterns of gene expression in cultured neural cells. These findings suggest that PTO-ODN can cause nonspecific effects on cell adhesion that could compromise interpretation of data from experiments using PTO-ODN.

The specificity by which DNA and RNA, as well as DNA/RNA binding proteins, can be targeted by exogenously administered ODN has proven to be a valuable tool for basic research in the fields of biology and medicine, and may also prove to be an effective approach for the treatment of disorders for which there are no effective drugs. The study of adhesion and migration in vivo is assisted by studies conducted in vitro. Adhesion of neurons in culture is achieved through the use of different extracellular matrix (ECM) components such as laminin and fibronectin, as well as cationic substances such as poly-ornithine, polyethylenimine (PEI), poly-D-lysine alone or in different combinations. We have recently observed that while using PTO-ODN aimed at

activating TLR9 (using CpG motif-containing ODN), embryonic neural cells grown on PEI but not other growth-facilitating compounds exhibited a detachment phenotype. During detachment, cell bodies of neurons aggregate and their axons form bundles (Fig. 1, and see Suppl. movie 1). The mechanism for this effect is still unknown; however, it is mediated at least in part by caspase-3.<sup>1</sup>

The phenotypic changes conferred by PTO-CpG in neurons plated on PEI are correlated with distinct transcriptional changes. Figure 2 shows that no overlap exists between mRNA transcripts that are either upregulated or downregulated following PTO-CpG treatment when compared to unmodified CpG. Moreover, PolyI:C, a TLR3 ligand that mimics viral RNA, induces changes that overlap with those of unmodified CpG. While different TLRs confer activation of distinct transcription factors, similar transcriptional changes are also expected to occur between different TLRs, as all TLRs can induce NFκB activation following pathogen infection. The fact that no overlap between PTO-CpG and unmodified CpG occurs, is another example for the non-specific effects conferred by PTO modification of the DNA backbone.

The ECM is a critical part of the nervous system and has been linked to many aspects of brain regulation from the development of the brain from a pool of neural stem/progenitor cells to the mature circuits formed by neurons and glia. Given the complexity of the nervous system, much of the work studying the ECM has been done in vitro. In the early 1980's, in vitro studies revealed the need to culture neurons on ECM substrates<sup>2,3</sup> and importantly, that they are also capable of making their own substrates (namely laminin, fibronectin, collagen IV).<sup>4,5</sup> Since these pioneering studies, many have relied on the use of these ECM substrates to maintain neuronal cultures.

The ECM also plays an equally critical role in the maintenance of neuronal adhesion in vivo. In the mammalian cortex, neurons are generated by asymmetric divisions of neural stem/precursor cells (also termed radial glia) at the ventricular wall. The resulting immature neurons then migrate along the radial glia fibers to the pre-plate where maturation occurs. This is a tightly regulated process that relies on a series of ECM interactions that are critical for the proper positioning and differentiation of cortical neurons. Early studies showed that during embryonic murine corticogenesis,

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Figure 1. PTO-CpG promotes neuronal cell body detachment from PEI-coated surfaces resulting in aggregates of cell bodies with long thin neurites extending between cell body aggregates. Cortical neurons plated on PEI coated dishes were treated with 10  $\mu$ M PTO-CpG and images of the same region were taken at 48-hour intervals.

the cortical pre-plate contains high levels of fibronectin and chondroitin sulfate proteoglycans.<sup>6</sup> We have recently shown that several laminin chains are also present in the pre-plate.<sup>7</sup> In addition, mutations in laminins or their integrin binding receptors result in a disruption of the cortical layering, which has been hypothesized to be linked to radial glia detachment (reviewed in ref. 8). The ECM also plays a critical role in neuronal maturation<sup>9</sup> and synaptic plasticity.<sup>10</sup> The examples discussed above underscore the importance of the ECM in regulating the nervous system in vivo, from early development to adulthood.

Our recent work suggests PTO-ODN may promote a detachment phenotype when neurons are cultured on PEI, laminin or poly-D-lysine but not poly-L-ornithine. Therefore, the use of this PTO-ODN and interpretation of experiments using this reagent may be confounded by the non-specific effect of the PTO-ODN on cell adhesion. Since different ECM components have the capability of not only hampering neuronal adhesion, but also differentiation and plasticity patterns in neurons, the relevance of this side effect to in vivo studies using PTO-ODN is even more complex, as a different milieu of ECM molecules regulate adhesion and signaling of neurons within the tissue.

Because PTO-ODN can have non-specific side effects on neuronal adhesion, changing the coating substance during in vitro studies might diminish or eliminate the non-specific effects of the PTO-ODN. Another approach would be to change the type of modification of the ODN. For example, it is interesting to study whether the conditions in which PTO-ODN induces detachment phenotype in neurons grown on PEI, apply also for ODN modified by other types of chemical modifications, such as locked-nucleic acids (LNA) or peptide-nucleic acids (PNA).

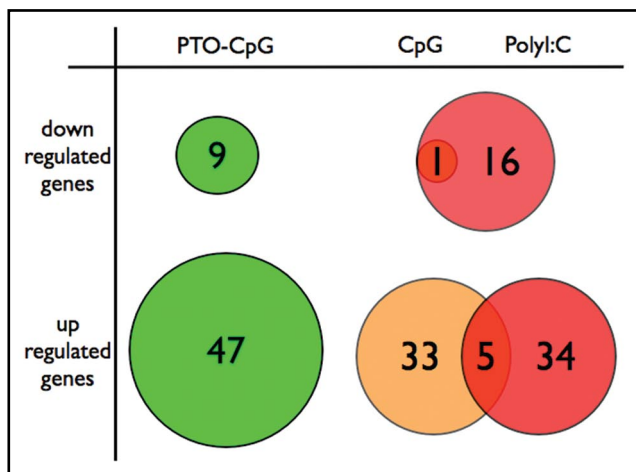
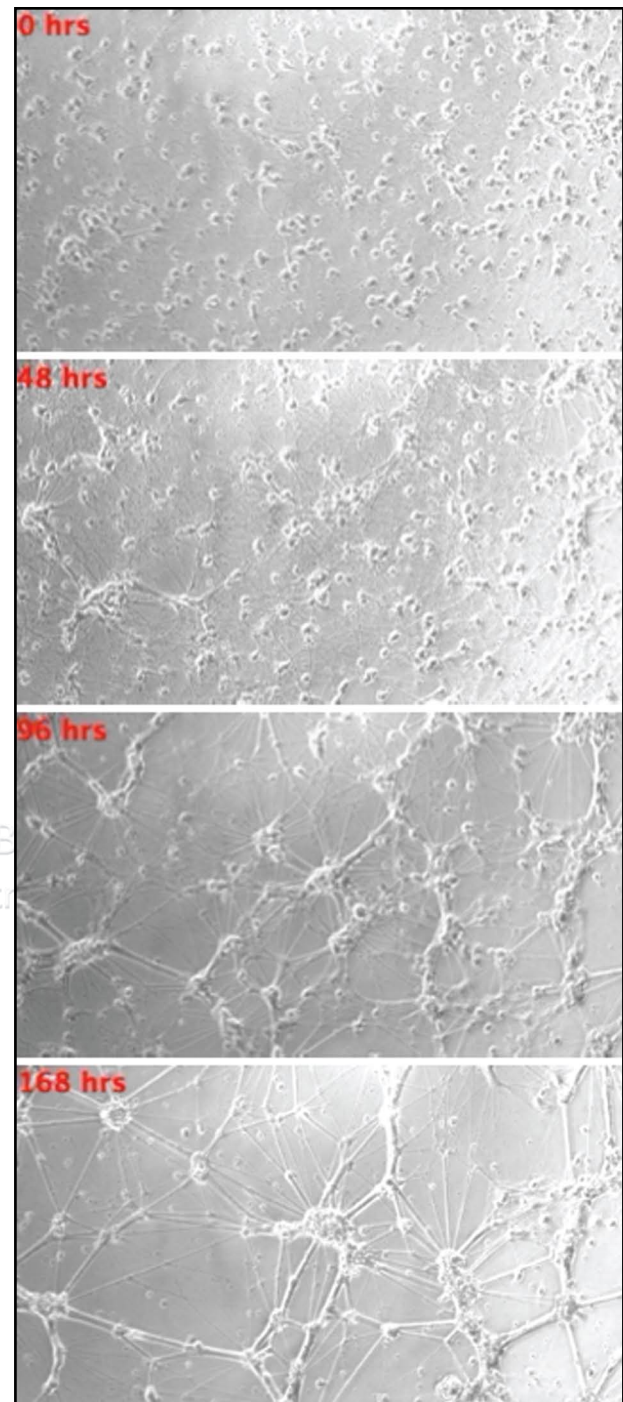


Figure 2. Transcriptional changes induced by CpG overlap with those induced by PolyI:C, but are distinct from the transcriptional changes induced by PTO-CpG. Cortical neurons that had been maintained in culture for 8 days were treated with 10  $\mu$ M CpG, 10  $\mu$ M PTO-CpG 10  $\mu$ g/ml PolyI:C or for 24 hours. RNA was then extracted and cDNA gene array was performed. For this analysis, only genes with a ( $\pm$ )1.9 fold change and a ( $\pm$ )3 fold z ratio change were considered. Genes overlapping between treatments are expressed as a venn diagram. The number inside each circle is the number of changed genes. Green spheres: PTO-CpG, orange spheres: CpG, red spheres: PolyI:C.

## Note

Supplementary materials can be found at:  
[www.landesbioscience.com/supplement/OkunCAM3-3-Sup.pdf](http://www.landesbioscience.com/supplement/OkunCAM3-3-Sup.pdf)

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