Common Cancer Drug Unexpectedly Influences Chromatin Dynamics at Promoters

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Most cancers respond to chemotherapeutic agents, with doxorubicin and other anthracyclines being particularly useful in controlling the proliferation of a wide variety of cancer cell types. The anti-cancer effects of doxorubicin appear to stem from its ability to interfere with the ability of topoisomerase II to relieve torsional stress in replicated DNA. The accumulation of resulting DNA double-strand breaks in doxorubicin-treated cells activates the DNA damage response and p53, an important tumor suppressor protein whose activity leads to cell death. However, whether the action of this important class of drugs affects other aspects of DNA-based processes has remained unknown. Given that doxorubicin interacts with DNA, Dr. Fan Yang and colleagues (from the laboratory of Dr. Steven Henikoff in the Basic Sciences Division) surmised that other aspects of DNA metabolism were potentially affected by the presence of this drug.

Chromosomal DNA is wrapped around histones to form nucleosomes, making the authors wonder whether the drug-DNA interaction alters the dynamics of these important DNA-packaging proteins. Specifically, Yang *et al.* wanted to study nucleosome turnover, which is an important aspect of chromatin structure and function. The authors had at their disposal the CATCH-IT method, which was previously designed in the Henikoff Lab for studying nucleosome turnover in cells. CATCH-IT involves labeling newly synthesized histones with a tag that can be used to purify labeled histone proteins from cell lysates to isolate histone-DNA complexes (*i.e.* to purify nucleosomes). DNA bound to histones is mapped by sequencing or microarray technologies, allowing precise temporal and spatial measurements of nucleosome turnover throughout the genome.

The investigators applied CATCH-IT to mouse squamous cell carcinoma (SCC) cell lines, probing nucleosome dynamics by hybridizing the DNA to high-density mouse promoter arrays. Genes were aligned by their transcriptional start sites (TSSs; for 5' end analysis) or transcriptional end sites (TESs; 3' end analysis), and the average nucleosome turnover was calculated in 50 bp intervals over a 6 kb range. Nucleosome turnover was highest at TSSs and slightly upstream of these sites. This was expected given the known labile nature of promoter-localized nucleosomes. Moreover, by using microarrays to obtain gene expression profiles from their CATCH-IT purifications, the authors

demonstrated that genes with the highest level of transcriptional output have the most rapid nucleosome turnover.

Yang *et al.* then treated the SCC cells with doxorubicin to ascertain whether the drug alters nucleosome turnover. Indeed, promoter regions exhibited increased nucleosome turnover at actively transcribed genes. Drug treatment had no effect on nucleosome dynamics at TESs, indicating the specific perturbation of chromatin structure at promoters. Further analysis revealed that ~43% of all annotated mouse genes showed increased nucleosome turnover at promoters after doxorubicin treatment but only a relatively minuscule two-fold maximal increase in gene expression.

Doxorubicin's major mode of action in chemotherapy involves activating the DNA damage response and p53 pathways to induce cell cycle arrest and death. To determine if either cellular response is involved in the doxorubicin-induced increase in nucleosome turnover, the authors applied the CATCH-IT technique to *ATM-/-* and *p53-/-* SCC cells. Surprisingly, the increased nucleosome turnover by doxorubicin required neither a functional DNA damage response (ATM) nor the activation of p53. These results reveal a novel mode of action of doxorubicin in influencing chromatin dynamics at promoters. This phenomenon is not restricted to doxorubicin, as another anthracycline, aclarubicin, also increased promoter-nucleosome turnover.

The work published by Yang and colleagues asks an important question: Are the accepted pharmacological actions of commonly used drugs well established, or do they also involve additional, albeit enigmatic, modes of action? The authors' results indicate that doxorubicin-related drugs increase the turnover of histones at promoters, potentially increasing the amount of time that promoter regions exist as "naked" DNA. This, combined with increased histone eviction and deposition (*i.e.* nucleosome turnover), may make promoter regions especially vulnerable to DNA breakage and might play a role in the anti-cancer effects of the anthracyclines. It is exciting to speculate that combining an anthracycline with a drug that targets chromatin modifying enzymes at promoters might enhance the anti-proliferative effects of many chemotherapeutics on cancer cells.

Yang F, Kemp CJ, Henikoff S. 2013. Doxorubicin enhances nucleosome turnover around promoters. *Curr Biol.* 23(9):782-7.

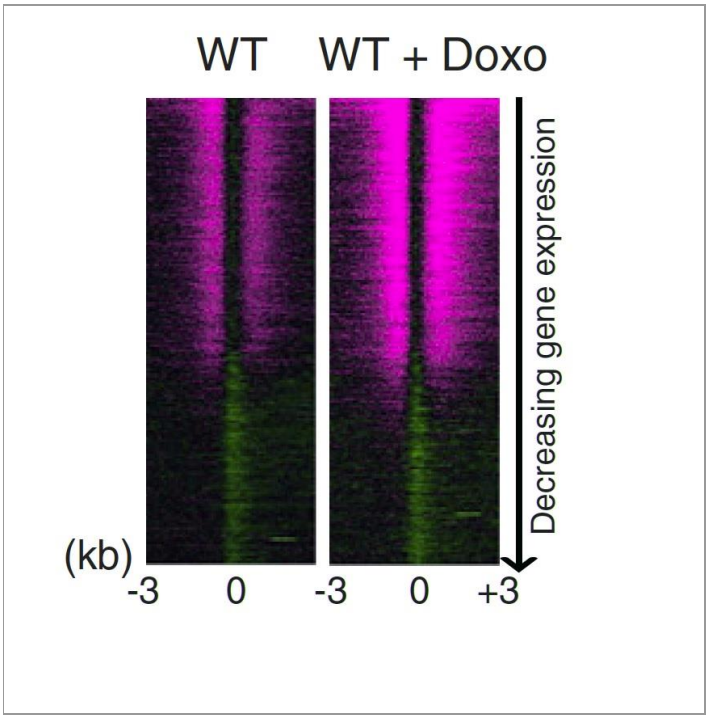

Image adapted from the manuscript

CATCH-IT data reveal increased turnover of promoter nucleosomes after doxorubicin treatment of SCC cells. The x-axis depicts the TSS (0) and 3 kb upstream (+3) and downstream (-3) of the TSS. Genes are ordered along the y-axis from highest (top) to lowest (bottom) gene expression. The depth of the purple color indicates the level of nucleosome turnover. Note the increased purple output in cells treated with doxorubicin, revealing increased nucleosome turnover in the vicinity of TSSs upon drug treatment.