Doxorubicin Gives Active Promoters a Break

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Doxorubicin is a widely used anticancer drug, and while its mechanism of action has been understood for decades, the reason for its selective toxicity to cancer cells has been the subject of much debate. Doxorubicin and related drugs, called anthracyclines, insert between DNA bases (intercalate) causing a positive torsion of the double helix. This alteration of DNA structure can trap enzymes including Topoisomerase II (TopoII), which relieves torsional stress by generating DNA double-strand breaks (DSBs) and subsequently ligating the cleaved strands. It has been shown that Doxorubicin traps TopoII following cleavage but before re-ligation, leading to cell killing, but it is not known if this, or overall inhibition of TopoII and subsequent failure to relieve torsional stress, is Doxorubicin's mechanism of selective cancer cell killing. Previously, postdoctoral fellow Dr. Fan Yang in the lab of Dr. Steven Henikoff (Basic Sciences Division), in collaboration with Dr. Christopher Kemp (Human Biology and Public Health Sciences Divisions), found that doxorubicin increases nucleosome turnover around active gene promoters (Yang *et al*., 2013). This led to the hypothesis that, by causing increased exposure of DNA through histone turnover, doxorubicin and other anthracyclines might cause increased levels of DNA DSBs within active promoters.

To map the positions of DSBs genome-wide, the authors used a protocol known as BLESS (direct in situ breaks labeling, enrichment on streptavidin and next-generation sequencing). They first performed BLESS in mouse squamous cell carcinoma (SCC) cells treated with various doses of the Doxorubicin as well as the TopoII poison Etoposide. Treatment of cells with a dose of Doxorubicin ten times below the therapeutic dose resulted in a slight increase in DSBs relative to untreated cells, while treatment of cells with therapeutic doses of Doxorubicin and Etoposide led to massive increases in DSBs. The authors then sequenced DNA from control cells, cells treated with Doxorubicin and Etoposide, and cells treated with a low dose of a second anthracycline, Aclarubicin, that does not poison TopoII.

Analysis of the distribution of spontaneous DSBs (that is, DSBs not caused by drug treatment) revealed preferential enrichment around gene promoters, with highly expressed genes displaying more DSBs. This suggested that spontaneous DSBs were coupled to gene transcription. Drug treatment resulted in increased levels of DSBs at promoters in a pattern similar to that seen in untreated cells, indicating that anthracyclines and Etoposide induce DSBs at regions of the genome that are susceptible to transcription-induced damage.

Mammalian promoters are frequently contained with CpG islands, which are regions of the genome containing a large proportion of C-G base pairs but low levels of DNA methylation. The authors thus asked if there were differences in DSB levels at CpG versus non-CpG. They first analyzed DSBs at all CpG islands and found that CpG islands were protected from DSBs regardless of their genomic location. Consistent with this observation, they found that CpG-island promoters displayed lower levels of DSBs than non-CpG promoters. Further analysis revealed that this distinction was not due to differences in sequence composition, and the authors thus concluded that CpG islands are protective against DSBs.

While analysis of DSB levels revealed increases relative to control with all drug treatments, it was not possible for the authors to compare between samples due to data normalization parameters. To address this, the authors used a 'spike-in' method, wherein a fixed amount of yeast DNA was added to each mouse sample. Using this approach, they found that both low and therapeutic doses of Doxorubicin produced equivalent increases of DSBs, while a therapeutic dose of Etoposide caused higher levels of DSBs than a low dose. Interestingly, a low dose of Aclarubicin caused even higher levels of DSBs than the therapeutic dose of Doxorubicin.

Combined with the authors' previous results, the work presented in this study indicates that anthracyclines cause increased nucleosome turnover at gene promoters, leading to increased DNA exposure and subsequent breakage. The similar patterns of DSBs induced by three drugs are striking, given that there are notable differences in their mechanisms: Doxorubicin intercalates into DNA and traps TopoII after DSB production, Etoposide traps TopoII and causes single-strand breaks, and Aclarubicin intercalates into DNA but does not trap TopoII. As all three drugs exert effects on DNA torsion, this is likely the common denominator in their similar effects on nucleosome turnover and DSB production.

Yang F, Kemp CJ, Henikoff S. 2015. Anthracyclines induce double-strand DNA breaks at active gene promoters. *Mutat Res* 773:9-15.

See also: Yang F, Kemp CJ, Henikoff S. 2013. Doxorubicin enhances nucleosome turnover around promoters. *Curr Biol*23(9):782–787.

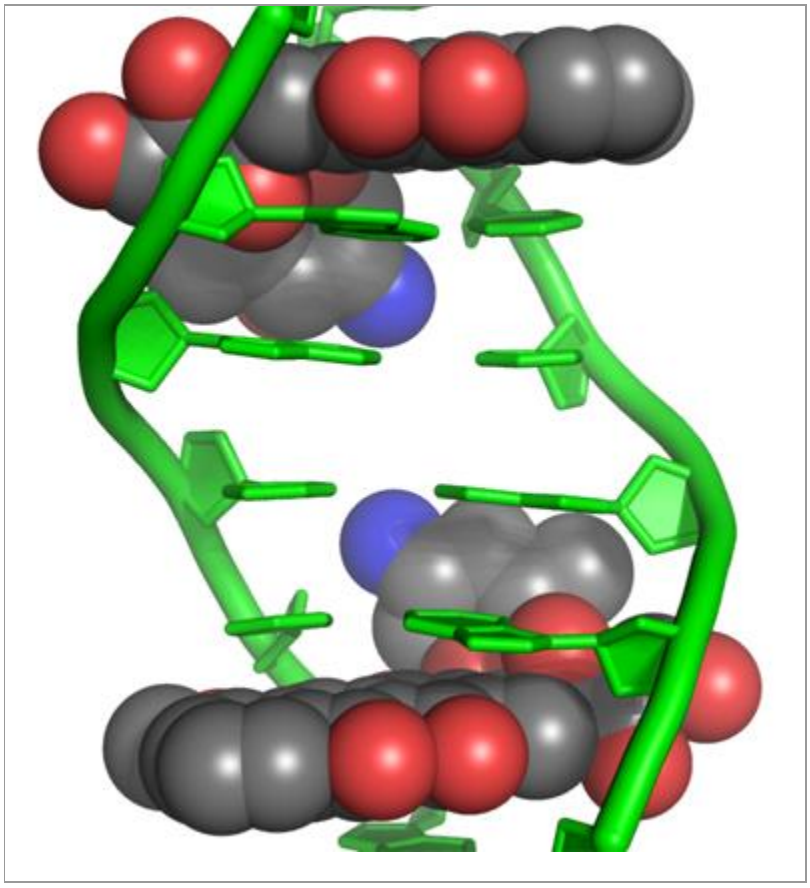

Image from Wikipedia

Structural representation of two Doxorubicin molecules intercalating into DNA.