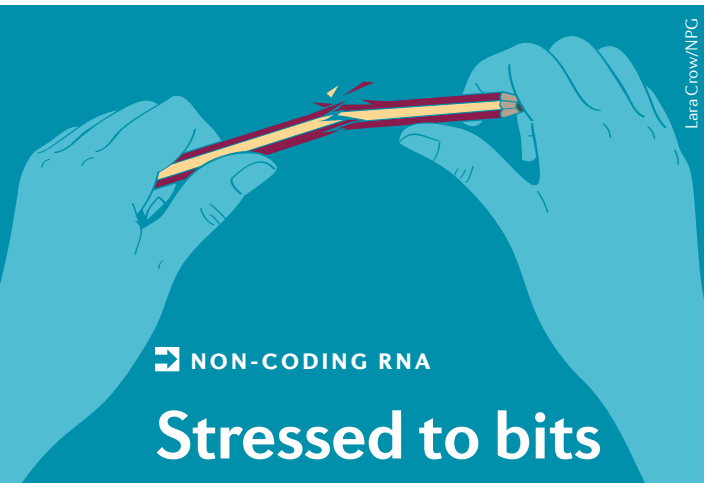

Stressed to bits

> hypoxic stress can activate a tRF- and YBX1-dependent tumour-suppressive mechanism

RNA fragments that arise from cleavage of tRNAs (tRNA-derived fragments (tRFs)) can be produced in response to stresses (such as hypoxia) and affect a variety of cellular functions. Goodarzi *et al.* hypothesized that these small non-coding RNAs might be involved in cancer progression.

Next-generation small-RNA sequencing of MDA-MB-231 breast cancer cells indicated that ~4% of the small-RNA population was composed of tRFs and that tRF levels rose significantly in hypoxic conditions. This increase in response to hypoxia was reduced in MDA-LM2 cells, which are a highly metastatic, *in vivo*-selected subpopulation of MDA-MB-231 cells. The tRFs induced by hypoxia contained a common linear sequence, suggesting that these might be bound by the same factor. Co-precipitation experiments with the tRF motif identified the RNA-binding protein YBX1. Endogenous YBX1 in MDA-MB-231 cells interacted with specific tRFs; this did not result from YBX1 interacting with abundant tRFs but instead seemed to be related to sequence specificity. In particular, fragments of $tRNA^{Glu}$, $tRNA^{Asp}$, $tRNA^{Gly}$ and $tRNA^{Tyr}$, which all share a common sequence motif, seemed to be most commonly bound to YBX1. Furthermore, quantitative binding analyses in cells transfected with synthetic tRF mimetics suggested that tRF^{Glu}, tRF^{Asp} and tRF^{Gly} compete for YBX1 binding.

YBX1 is known to regulate both mRNA translation and transcript stability. Several lines of evidence were consistent with a model in which tRF^{Glu}, tRF^{Asp}, tRF^{Gly} and tRF^{Tyr} displace transcripts from YBX1, leading to transcript instability. Several specific target transcripts that had strong *in vivo* interactions with YBX1 via their 3′ untranslated regions were stabilized in a YBX1-dependent manner by tRF knockdown using antisense locked nucleic acids (LNAs). Conversely, these transcripts were depleted from YBX1 immunoprecipitations following transfection of tRF mimetics.

YBX1 is overexpressed in many cancers and can promote metastasis, and many transcripts modulated by tRFs and YBX1 are known to promote tumour progression and metastasis. Therefore, the authors reasoned that these tRFs, which inhibit YBX1 activity on target transcripts, might suppress tumour progression. Transfection of LNAs targeting tRFs enhanced *in vitro* invasion of MDA-MB-231 cells and CN34 breast cancer cells; conversely, transfection of tRF mimetics into MDA-LM2 cells or metastatic CN-LM1a cells suppressed invasion in a YBX1-dependent manner. Furthermore, levels of tRF^{Glu}, tRF^{Asp} and tRF^{Gly} in non-metastatic primary breast tumours were significantly higher than in metastatic tumours.

In hypoxic conditions, levels of tRF^{Glu}, tRF^{Asp} and tRF^{Gly} increased in MDA-MB-231 cells, and YBX1 target transcripts were downregulated, but this did not occur in MDA-LM2 cells. Tail-vein injection of MDA-MB-231 cells carrying a hypoxia reporter confirmed that these cells experience hypoxia early upon reaching the lungs, and the expression of tRF target genes was reduced in these cells. Consistent with *in vitro* data, LNAs against tRFs increased metastatic colonization of the lungs by MDA-MB-231 and CN34 cells, whereas tRF mimetics reduced colonization by MDA-LM2 and CN-LM1a cells in a YBX1-dependent manner.

These data support the hypothesis that hypoxic stress can activate a tRF- and YBX1-dependent tumour-suppressive mechanism *in vivo* and that this is likely to be circumvented in metastatic cells through upregulation of YBX1 and suppression of tRF generation.

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ORIGINAL RESEARCH PAPER Goodarzi, H. *et al.* Endogenous tRNA-derived fragments suppress breast cancer progression via YBX1 displacement. *Cell* **161**, 790–802 (2015)