The oscillatory expression of ERα as a new drug target for resensitizing hormone-refractory breast cancer

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Endocrinetherapies such as tamoxifen and aromatase inhibitors are the standard treatment options for estrogen receptor (ER)-positivebreast cancer patients. However about one-third of patients do not benefit from therapies and they develop recurrent disease. Resistance to endocrine therapies, thus, remain a major challenge in providing effective treatments for these patients. Potential mechanisms of resistance to endocrine therapies have been identified, often involving enhanced expression or increased activity of ERα. Our laboratory has demonstrated that a well described polymorphic sequence in the first intron of *ER*α (*Pvu*II and *Xba*I) has a key role in regulating the receptor content in proliferating cells. We have shown that the RNA Pol II elongation is blocked at the polymorphic site and that modulates the release of the pausing polymerase (Vantaggiato et al., 2014). The aim of the present study was to investigated the role of transcriptional attenuation in the onset of hormone refractory breast cancers. Analysis of 36 human breast cancer cases demonstrated that the mechanism is altered during breast cancer progression and contributes to the establishment of a hormone refractory phenotype highlighting the clinical relevance of the Pol II pausing at the ERα intronic site. The application of several different experimental approaches (run on, real-time PCR, reporter assay, northern, western and immunohistochemistry) allowed us to conclude that the RNA pol II block is cell-cycle dependent and is released during G2 phase leading to a peak of receptor expression in G1. In this work we have demonstrated that the polymorphic site at the intron 1 of *ERα* is necessary for the pulsatile activity of the receptor during cell cycle. Most interestingly, we here provide evidence of the involvement of attenuation in the switch from ER-positive to ER-negative tumours during the disease progression, thus suggesting the relevance of this mechanism in the tumor progression towards a hormone refractory phenotype where the blockage of Pol II elongation contributes to turn off ERα synthesis. To dissect the signalling pathways involved in the formation of these ER-negative cancers, we investigate the attenuation mechanism in the triple negative breast cancer cell line MDAMB 468. We show that the *ERα* promoter is fully active, while a full length mRNA is not produced because the Pol II elongation is impaired at the pausing site. Based on literature data, we have tested several signalling pathways/transcription factors to evaluate their activity in the block of Pol II elongation. Our study identify Il-4 signalling and c-MYB as key nodes of the signalling pathways modulating the release of the polymerase from the intronic block, thus suggesting a novel strategy to re-sensitize hormone refractory breast cancers.