Role of DNA methylation in INK4a-ARF-INK4b locus expression in breast cancer

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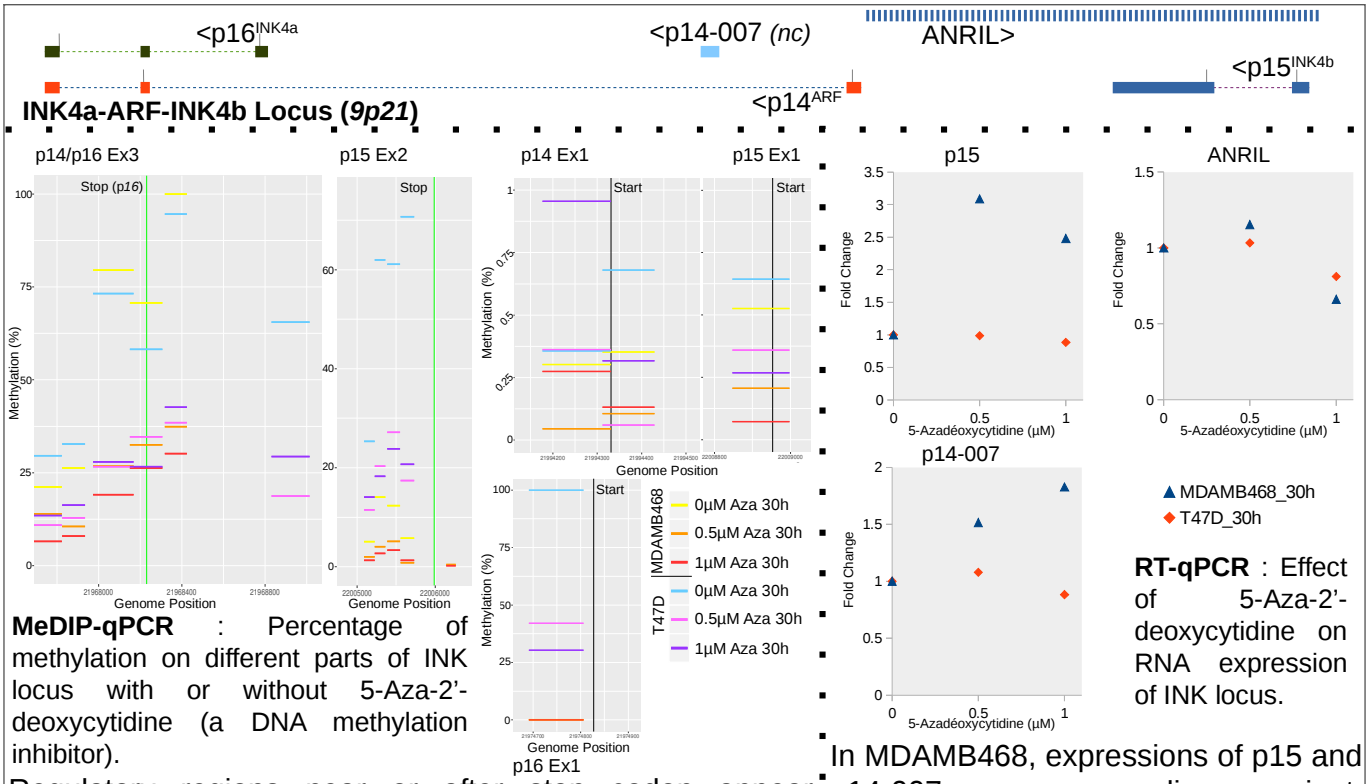
Introduction

More than 75% of breast cancers are ER+. The CDK4/6 inhibitors, having the same role of endogenous p16/p15, associated with hormonotherapy appear as new standard treatment for metastatic tumors. The INK locus encodes p16/p14-p15 proteins, cell cycle regulators, and ANRIL, a ncRNA, which are often altered in cancers. In this study, we explore the role of DNA methylation of this locus in expression regulation of p16/p14-p15 and ncRNAs.

Results



MeDIP-qPCR : Percentage of methylation on different parts of INK locus with or without 5-Aza-2'-deoxycytidine (a DNA methylation inhibitor).

Regulatory regions near or after stop codon appear hypermethylated. Conversely, regions around gene promotors, often described as hypermethylated in cancers, have a low methylation rate (<1%) except for p16 in T47D.

RT-qPCR : Effect of 5-Aza-2'-deoxycytidine on RNA expression of INK locus.

In MDAMB468, expressions of p15 and p14-007, a non-coding variant, increase while ANRIL decreases. Expressions of p14/p16 are constant no matter treatment in both cell lines. *(Not shown)*

When methylation, already low for p14/p15 Exon1, decreases below 5% after stop codon of p15, it lead to its overexpression. But, even after treatment, a methylation rate over 20% is not associated with expression modifications.

Conclusion

MDAMB468 (triple negative), and T47D (ER+), show different methylation and expression profiles. We know that ANRIL and methylation on gene promotors have an effect on locus silencing but DNA methylation around or after the stop codon of a gene are poorly described in litterature but they seem to be also a role in RNA expression regulation.

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