ABSTRACT

Breast cancer is a highly heterogeneous disease classified clinically by expression of estrogen receptor alpha ERα, progesterone receptor, and human epidermal growth factor receptor. Molecular expression profiling identified a luminal breast cancer sub-type that can be sub-divided into luminal A and B. Compared to luminal A, luminal B tumors have increased proliferation, poor prognosis, endocrine therapy resistance, and complex genomes, including amplification of the 8p11-p12 genomic region. This amplicon occurs in 15% of primary breast tumors, correlates with poor prognosis and tamoxifen resistance, and harbors several oncogenes. Two of these oncogenes, ASH2L and NSD3 (WHSC1L1), promote transcription via epigenetic modification of histone proteins. NSD3 has a long isoform that is associated with di-methylation of lysine 36 on histone 3 (H3K36me2) and a short isoform that lacks a catalytic SET domain but retains the ability to interact with chromatin. ASH2L also lacks a catalytic SET domain yet is tightly and specifically linked to tri-methylation of lysine 4 on histone 3 (H3K4me3) in gene promoters. In this study, we tested the hypothesis that ASH2L and NSD3 cooperate to regulate expression of a suite of genes important in breast cancer, including ESR1, which encodes ERα. We discovered that NSD3-short is the major oncogenic isoform of NSD3 and its amplification and overexpression leads to overexpression and estrogen-independent activation of ERα. We also demonstrated that knockdown of ASH2L reduces H3K4me3 specifically in promoters of genes important to cell cycle progression. ASH2L also regulates promoter H3K4me3 at NSD3 and expression of both NSD3 and ERα. Knockdown of ASH2L reduced sensitivity to the cell cycle inhibitor palbociclib in the 8p11-p12 amplicon-bearing SUM-44 cell line. Together, the data presented here identify a role for ASH2L and NSD3 in cooperative regulation of genes important to cell
cycle regulation, including ESR1, and demonstrate that ERα is active in an estrogen-independent manner in the context of overexpression of these oncogenes. We have discovered a novel mechanism of endocrine resistance in luminal B breast cancers and provided evidence for the 8p11-p12 amplicon as a biomarker of patients who will respond to cell cycle inhibitors and epigenetic therapies against histone methyltransferase enzymes.