Deciphering the transcriptional complex critical for epigenetic regulation driven by endothelin A receptor/β-arrestin-1 in ovarian cancer

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Current evidence highlights the ability of scaffold proteins, such as □-arrestin-1 (ARRB1), to create signalling platforms that drive epithelial ovarian cancer (EOC) progression upon endothelin A receptor (ET_A) activation. To decipher the transcriptional core complex that is governed by endothelin -1 (ET-1), here we dissected the yet uncovered function of ARRB1 as nucleus chaperone in mediating ET_A-dependent □-catenin nuclear responses. As shown by confocal and biochemical analyses, ET-1/ET_A activation induces nuclear translocation of both ARRB1 and □-catenin and their co-localization, which were impaired by introduction of nuclear export signal by a single point (Q394L) mutation in ARRB1. Furthermore, ET-1-dependent ARRB1 nuclear translocation correlated with an increase in □-catenin transcriptional activity (n=6, p<0.001) and in the enhanced expression of □-catenin target genes involved in tumor progression, such as ET-1, matrix-metalloproteinase (MMP)-2 and Cyclin D1. These effects were inhibited by ARRB1 silencing and rescued by the expression of ARRB1 wild type (WT) but not ARRB1-Q394L, confirming that ARRB1 is necessary for ET-1-driven β-catenin/TCF4 transcriptional activity. A thorough characterization of the molecular events by which A□□□1 contributes to regulation of these genes revealed that ARRB1 and □-catenin participate in the formation of a nuclear complex on the promoter regions of these genes in ET_A-dependent manner, as shown by ChIP (n=6) and IP assays (n=3). This nuclear complex includes the histone acetyltransferase p300 on these promoters to increase H3 and H4 hyperacetylation and the reorganization of chromatin, thereby increasing gene expression. In an i.p. model of human EOC metastasis in mice, transfection of EOC cells with sh-ARRB1 or ARRB1-Q394L significantly reduced the numbers of metastatic nodules compared to control (3,2±0,4 and 3,6±0,3 versus 14±2, n=3, p<0,001). Furthermore, ChIP assays on the metastatic nodules showed reduced association of □-catenin and ARRB1 on ET-1 and MMP-2 promoters in nodules from cells lacking ARRB1 or expressing ARRB-1Q394L compared to control (n=3). Overall, our study provides for the first time a new mode by which ET_A may regulate gene transcription achieved via a nuclear interaction connecting ET_A and ARRB1 to □-catenin transcriptional activity and metastatic behaviour in EOC cells. Considering the milieu of transcriptional proteins, distinct ARRB1-complexes can recruit or interact with a variety of factors that modulate the transcription of specific target genes, such as ET-1. Altogether these findings reveal a positive inter-regulation between β-catenin and ET-1 that amplify the ET-1/ET_A autocrine loop in EOC cells, orchestrating the network regulating the fine tuning of EOC metastasis.

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