Abstract

Study question: Is the DNA damage response (DDR) dysregulated in the eutopic endometrium of women with endometriosis?

Summary answer: Endometrial expression of genes involved in DDR is modulated in women with endometriosis, compared to those without the disease.

What is known already: Ectopic endometriotic lesions are reported to harbour somatic mutations, thereby hinting at dysregulation of DDR and DNA repair pathways. However, it remains inconclusive whether the eutopic endometrium also manifests dysregulated DDR in endometriosis.

Study design, size, duration: For this case-control study conducted between 2015 and 2019, eutopic endometrial (E) samples (EE- from women with endometriosis, CE- from women without endometriosis) were collected in either mid-proliferative (EE-MP, n = 23; CE-MP, n = 17) or mid-secretory (EE-MS, n = 17; CE-MS, n = 9) phases of the menstrual cycle. This study compares: (i) DNA damage marker localization, (ii) expression of DDR genes and (iii) expression of DNA repair genes in eutopic endometrial samples from women with and without endometriosis.

Participants/materials, setting, methods: The study included (i) 40 women (aged 31.9 ± 0.81 years) with endometriosis and (ii) 26 control women (aged 31.4 ± 1.02 years) without endometriosis. Eutopic endometrial samples from the two groups were divided into different parts for histological analysis, immunohistochemistry, RNA extraction, protein extraction and comet assays. Eighty-four genes of relevance in the DNA damage signalling pathway were evaluated for their expression in eutopic endometrial samples, using RT2 Profiler PCR arrays. Validations of the expression of two GADD (Growth Arrest DNA Damage Inducible) proteins - GADD45A and GADD45G were carried out by immunoblotting. DNA damage was assessed by immunohistochemical localization of γ-H2AFX (a phosphorylated variant of histone H2AX) and 8-OHdG (8-hydroxy-2'-deoxyguanosine). RNA sequencing data from mid-proliferative (EE-MP, n = 4; CE-MP, n = 3) and mid-secretory phase (EE-MS and CE-MS, n = 4 each) endometrial samples were scanned to compare the expression status of all the genes implicated in human DNA repair. PCNA (Proliferating Cell Nuclear Antigen) expression was determined to assess endometrial proliferation. Residual DNA damage in primary endometrial cells was checked by comet assays. Public datasets were also scanned for the expression of DDR and DNA repair genes as our RNASeq data were limited by small sample size. All the comparisons were made between phase-matched endometrial samples from women with and without endometriosis.

Main results and the role of chance: Endometrial expression of DDR genes and intensity of immunolocalized γ-H2AFX were significantly ($P < 0.05$) higher in EE, compared to CE samples. DDR proteins, especially those belonging to the GADD family, were found to be differentially abundant in EE, as compared to CE. These patterns were evident in both mid-proliferative and mid-secretory phases. Intriguingly, higher DDR was associated with increased cell proliferation in EE-MP, compared to CE-MP. Furthermore, among the differentially expressed transcripts (DETs) encoded by DNA repair genes, the majority showed up-regulation in EE-MP, compared to CE-MP. Interestingly, CE-MP and EE-MP had a comparable percentage ($P > 0.05$) of cells with residual DNA damage. However, unlike the mid-proliferative phase data, many DETs encoded by DNA repair genes were down-regulated in EE-MS, compared to CE-MS. An analysis of the phase-matched control and endometriosis samples included in

the GSE51981 dataset available in the Gene Expression Omnibus database also revealed significant ($P < 0.05$) alterations in the expression of DDR and DNA repair genes in EE, compared to CE.

Large-scale data: N/A.

Limitations, reasons for caution: The study was conducted on a limited number of endometrial samples. Also, the study does not reveal the causes underlying dysregulated DDR in the eutopic endometrium of women with endometriosis.

Wider implications of the findings: Alterations in the expression of DDR and DNA repair genes indirectly suggest that eutopic endometrium, as compared to its healthy counterpart, encounters DNA damage-inducing stimuli, either of higher strength or for longer duration in endometriosis. It will be worthwhile to identify the nature of such stimuli and also explore the role of higher genomic insults and dysregulated DDR/DNA repair in the origin and/or progression of endometriosis.