Disease Models & Mechanisms 4, 139-140 (2011) doi:10.1242/dmm.007278

Progression of endometriosis to cancer: too MUCh FoxP3$^+$ regulatory T-cell response?

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Endometriosis, a major contributor to pelvic pain and subfertility, affects 6-10% of women of reproductive age and is characterized by endometrial-like tissue outside the uterus, mainly in the pelvic peritoneum and ovaries (Giudice, 2010). It is becoming widely accepted that endometriosis, especially ovarian endometriotic cysts, is a neoplastic disease and, consequently, might have malignant potential (Mandai et al., 2009). However, the pathological mechanisms underlying the development and maintenance of endometriosis, and its progression to cancer, remain to be elucidated. It is known that adaptive (T and B cells) and innate [including natural killer (NK) cells] immunity are both important for preventing primary tumour formation; thus, in order to progress to advanced stages, tumours must be able to evade or suppress immunity (Cao, 2010). Therefore, understanding the mechanisms of tumour tolerance is a major challenge for cancer research.

Various molecular alterations have been linked to the malignant transformation of endometriosis: hyperestrogenism, changes in the expression of cytokines, and mutation or loss of function of the tumour suppressor genes *p53*, *Kras* and *PTEN* (Mandai et al., 2009). The relevance of the genetic alterations in *Kras* and *PTEN* has been further supported by an elegant mouse model involving Cre-*loxP* recombination in *loxP*-Stop-*lox*P-*Kras*$^{G12D/+}$ (Kras) mice, in which forced expression of oncogenic *Kras* in ovarian surface epithelium results in ectopic endometrial-like morphology. Furthermore, the combination of both *Kras* expression and conditional *PTEN* deletion gives rise to ovarian carcinoma (Dinulescu et al., 2005).

Genetic and epigenetic changes in tumour cells also induce the expression of antigens (Cao, 2010), such as MUC1, a membrane-bound mucin that is normally expressed in most epithelial cells but is overexpressed and aberrantly glycosylated in various carcinomas (Bafna et al., 2010), including in ovarian cancer (Van Elssen et al., 2010). It has also been proposed that MUC1 contributes to growth and survival of cancer cells by stimulating estrogen-receptor-α-mediated transcription (Bafna et al., 2010). Therefore, MUC1 is a potential target for immunotherapy strategies to treat ovarian cancer. However, the immunogenic properties of MUC1 in precursor lesions such as endometriosis have not been extensively explored. In a previous issue of *Disease Models & Mechanisms*, Budiu et al. (Budiu et al., 2009) investigated this topic by studying the antigen-specific immune response induced by the expression of human MUC1 in the Kras conditional mouse model of endometriosis (discussed above). The novel model, named MUC1Kras, was generated by crossing Kras mice with transgenic mice expressing human *MUC1* under its endogenous promoter (Budiu et al., 2009).

The authors first validated the use of MUC1 as a marker for human endometriosis: MUC1 glycoprotein was found to be normally expressed on epithelial cells of eutopic endometrial glands in human uterus and, notably, ectopic (endometriotic) glands in ovaries were also MUC1 positive (MUC1$^+$). Quantitative PCR confirmed that samples of ovarian tissue from patients with ovarian endometriosis exhibited increased levels of *MUC1* mRNA compared with samples obtained from normal ovaries (see figure 1 in Budiu et al., 2009).

Next, the authors examined the pattern of MUC1 expression in the MUC1Kras mouse strain. The distribution of human MUC1 protein throughout the gynaecological tract of healthy (i.e. unexposed to Cre recombinase) MUC1Kras female mice closely resembled that observed in healthy humans (see figure 2 in Budiu et al., 2009). Delivery of recombinant adenovirus for targeted expression of Cre recombinase (AdCre) in the ovarian bursa (capsule) of MUC1Kras mice resulted in ovarian endometriotic lesions after 32 weeks (at 12 and 24 weeks, lesions were not detected), as previously observed in Kras mice (Dinulescu et al., 2005). Estrogen receptor and cytokeratin 7, which are typical markers used to diagnose endometriosis, were also detected in ovarian lesions by histological staining. Importantly, lesions were also MUC1$^+$ (see figures 3 and 4 in Budiu et al., 2009). At 32 weeks post-AdCre-injection, when ovarian endometriotic lesions had developed, increased expression of MUC1 was detected in both ovarian and bursal epithelial cells, suggesting that the level of expression of MUC1 positively correlates with the development of disease in MUC1Kras mice (see figure 5 in Budiu et al., 2009).

The authors then studied the immunogenicity of MUC1 during disease development. They first assessed the humoral (B cell) response to MUC1. Blood samples were collected at 12 and 32 weeks after AdCre injection, and the presence of antibodies against MUC1 was determined and compared with baseline antibody levels. Although no changes were found in the Kras mice at any time point, an increased humoral immune response specific for the MUC1 antigen was found in both MUC1 and MUC1Kras mice. When comparing these two strains, titers of MUC1-specific IgM antibodies were found to be higher in MUC1Kras mice than in MUC1 mice at both 12 and 32 weeks. Conversely, anti-MUC1 IgG titers in MUC1Kras mice significantly increased only after 32 weeks, when IgM titers decreased, reflecting an IgM-to-IgG isotype switch (see figure 6 in Budiu et al., 2009).

Although effector T cells, B cells and NK cells are known to be involved in anti-tumour

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immunity, recent studies have implicated regulatory T cells in inducing tolerance to tumours (Cao, 2010). Initially associated with the control of autoimmune responses (Sakaguchi et al., 1995), regulatory T cells (T_{Reg} cells) – a subset of $CD4^+$ T cells expressing the transcription factor forkhead box P3 (FoxP3) – were subsequently recognized to limit essentially all types of adaptive immune responses (Sakaguchi et al., 2010). When Budiu et al. analyzed T cell populations in MUC1Kras mice, they found an increase in the percentage of $CD4^+FoxP3^+$ T_{Reg} cells in the draining lymph nodes of both MUC1Kras and Kras diseased mice (at 32 weeks) compared with uninjected MUC1Kras controls (Budiu et al., 2009). Analyses of spleen cells also showed an increase in the percentage of T_{Reg} cells in the diseased mice, whereas the percentage of interferon-γ-producing splenic T cells after polyclonal stimulation (an indicator of anti-tumour activity) was lower in MUC1Kras diseased mice than in healthy age-matched MUC1Kras mice. Importantly, these changes were only detected after 32 weeks, when detectable lesions had developed in MUC1Kras and Kras mice. Finally, and most importantly, the authors reported that *FOXP3* mRNA levels were higher in tissue samples from patients with endometriosis than in samples from healthy controls, suggesting an increase in the size of the T_{Reg} cell population in endometriosis. Consistent with this, flow cytometry analysis of the $CD4^+$ T cells infiltrating the ovaries of two patients with endometriosis showed that they had higher proportions of T_{Reg} cells at the site of lesions, but not in the peripheral blood (see figure 7 in Budiu et al., 2009).

Data from Budiu et al. (Budiu et al., 2009) and more recently from Berbic et al. (Berbic et al., 2010) uncover an association between altered levels of T_{Reg} cells and endometriosis. Given the apparent increase in the size of the T_{Reg} cell population at the site of lesions in both diseased MUC1Kras mice and patients with endometriosis, it is plausible that downregulation of local immune responses by a T_{Reg}-cell-dependent mechanism could underlie deficient clearing of lesions. However, the antigen specificity and origin of these T_{Reg} cells remain to be clarified. T_{Reg} cells can be generated in the thymus (referred to as naturally occurring T_{Reg} cells) or in response to specific microenvironments (referred to as induced T_{Reg} cells) (Sakaguchi et al., 2010). In addition, T_{Reg} cells proliferate in response to external signals such as those provided during antigen presentation by mature dendritic cells (Walker, 2004). Notably, physiological levels of estradiol, such as would be present in ovarian lesions, have been shown to enhance the proliferation of human T_{Reg} cells responding to T-cell-receptor engagement (Prieto and Rosenstein, 2006). It is thus possible that estradiol promotes the onset of immunological tolerance to MUC1 once it is overexpressed during endometriosis development through expanding a pre-existing pool of MUC1-specific T_{Reg} cells. Given that MUC1 is an ovarian-tumour-associated antigen (Van Elssen et al., 2010), this hypothesis predicts that local expansion of T_{Reg} cells that are specific for MUC1 might suppress anti-tumour responses and facilitate the progression of endometriosis to ovarian cancer in susceptible women. Interestingly, tolerance to tumour antigen, causing general cytotoxic T cell unresponsiveness, has been reported to occur at the premalignant stage in a transgenic mouse model of sporadic cancer, despite the production of tumour-specific antibodies; this was shown to be a condition that was permissive for cancer progression (Willimsky et al., 2008). However, whether this tolerance is mediated by T_{Reg} cells remains to be investigated.

The MUC1Kras mice reported in this study combine the advantages of previous endometriosis animal models – non-human primates (that closely parallel human endometriosis) and heterologous transplanted mice (that allow different experimental approaches at lower cost but are immunosuppressed) (Tirado-Gonzalez et al., 2010) – to investigate the role of immune suppression in endometriosis and ovarian cancer. Furthermore, MUC1Kras mice offer a valuable in vivo system in which to evaluate preventive and therapeutic strategies for endometriosis and ovarian cancer. Because estradiol boosts the proliferation of T_{Reg} cells, MUC1Kras mice could be used to test the potential benefits of the local delivery of anti-estrogenic compounds to prevent ovarian cancer development in endometriosis patients.

ACKNOWLEDGEMENTS

Helpful comments from Yvonne Rosenstein and Carl W. Cotman guided this manuscript.

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