Chapter 9
General Discussion

9 General Discussion

The classical NFκβ pathway begins with TNF-α and is mediated by proteins that compose the IKK complex that aid in cell survival mechanisms. In this study we were concerned whether NFκβ was activated via the IKKα morphogenetic pathway or the IKKβ in the pro-inflammatory pathway (Figure 9.1) in endometriosis. We also wanted to discover whether differences in proteasome and Iκβα exists within eutopic and ectopic endometrial cells, to give us an indication of whether NFκβ is the pathway involved in cell survival, or that another mechanism located more upstream is potentially responsible.

The molecular studies of human tissues reveal that a trend toward a high fold change in NFκβ and PA28 between the eutopic and ectopic endometrium exists but this result was not statistically significant. IKKα is possibly not the kinase responsible for any NFκβ mediated endometrial cell survival in endometriosis in women. However, the quality and quantity of RNA extracted from tissues collected from many women, we could not conduct verification experiments on a larger patient cohort. This needs to be examined in the future to definitively rule out IKKα's role in women with the condition.

In the molecular studies of baboon tissues, we discuss the need for future investigations to include any fold change studies between the eutopic and ectopic endometrium in a larger cohort of lyophilised baboon tissues with suitable RNA quality and quantity. However, since NFκβ is not present in ectopic endometrial tissues of baboons, mimicking the result found in humans in Chapter 3, another protein pathway activated by ubiquitin, that is also involved in cell survival such as the JNK pathway may be a worthwhile mechanism to explore.

Immunohistochemical studies of eutopic baboon endometrial tissues show that IKKα is a likely candidate for endometrial cell survival in baboons, but this is likely to be

modulated through a mechanism other than the ubiquitin mediated NFκβ pathway. A similar level in ubiquitin and NFκβ immunostaining suggests that ubiquitin may stabilize tagged proteins for various functions instead of proteasomal degradation that allows the continual association of Iκβα with NFκβ. This union prevents the transcription of NFκβ survival factors in the baboon. A similar result was found within ectopic endometrial tissues of the baboon.

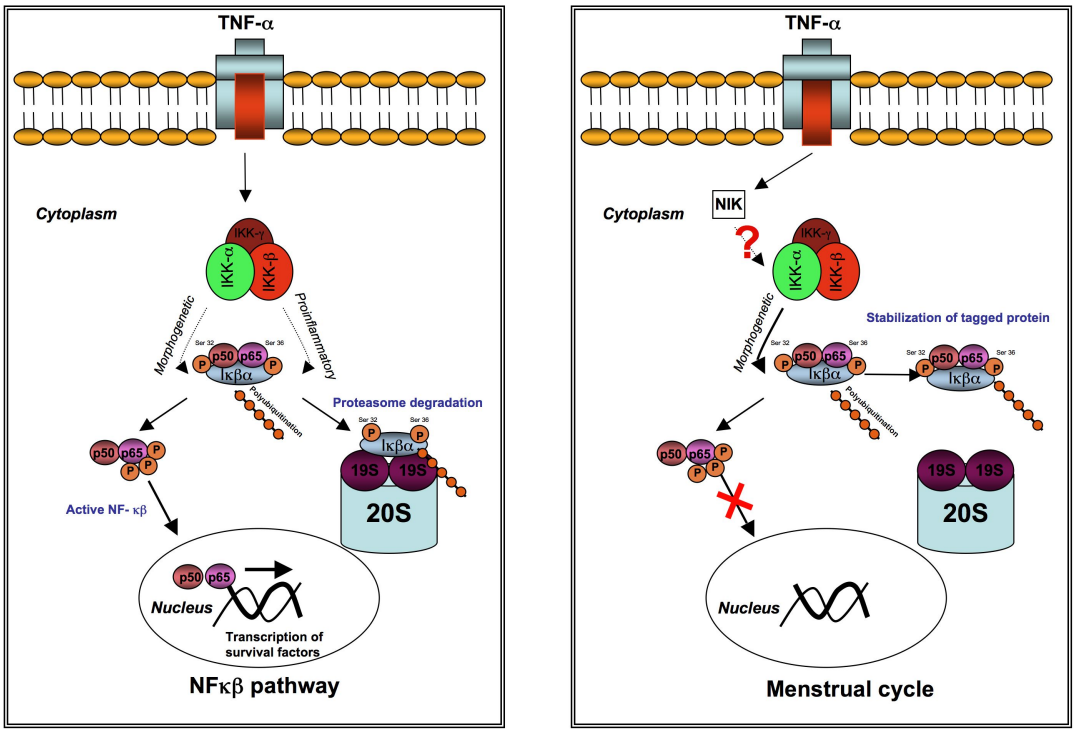

Figure 9.1: (A) The classical NFκβ pathway; (B) proposed mechanism during the menstrual cycle.

A proposed model on the involvement of IKKα in baboons is illustrated on Figure 9.1B, that is in contrast to the classical model of Figure 9.1A. Perhaps, as Ling and colleagues suggested, an undefined activator of IKKα, such as NIK (Ling *et al*, 1998)

located upstream of the ubiquitin- NFκβ preferentially phosphorylates p65 in the baboon.

Our protein study suggests that the NFκβ pathway is likely not responsible for endometrial cell differentiation between the proliferative and secretory phase, due to the similar IKKα, IKKβ and Iκβα levels observed in the eutopic human endometrium. The elevated 19S proteasome however, may allow an increased ability to recognize regulatory proteins that controls endometrial cell differentiation particularly within the nucleus of stromal cells at secretory phase. Whilst in the ectopic endometrium a continued association between proteins such as Iκβα and NFκβ exists due to the low 19S protein levels during secretory phase, contrasting the findings at the proliferative phase, where a higher 19S protein recognition exist.

Our data reveals that neither IKKα nor IKKβ would have a greater phosphorylating potential of the p65 subunit of NFκβ on Ser-536. Furthermore, IKKβ is definitively not the kinase involved in phosphorylating Ser-536 on NFκβ in endometriosis, as this was absent within the ectopic tissue during secretory phase. This result was statistically significant in comparison to the eutopic endometrium. The similar levels of Iκβα and the absence of NFκβ immunostaining within eutopic and endometriotic tissues indicate that the NFκβ pathway is not responsible for any survival potential of endometriotic tissues in our patient cohort.

In conclusion, we have observed that IKKα is a likely candidate protein for ectopic endometrial cell survival in the baboon. A larger patient cohort is needed to definitively ascertain the role of IKKα in women. It is likely that a different mechanism and not the ubiquitin mediated NFκβ pathway are responsible for ectopic cell survival in baboons and women with endometriosis.

In the future, molecular and immunohistochemical studies on a larger cohort of women and baboons is essential to accurately ascertain any changes in the ubiquitin- NFκβ

pathway. NIK is a worthwhile upstream protein to investigate for it is able to activate IKKα in the morphogenetic pathway. Meanwhile the cell survival capacity of the JNK pathway may also be a worthwhile mechanism to explore in the future.

As all of the current studies are correlative/observational in nature, it is important to also use a mechanistic approach using endometrial cells lines with oestrogen and progesterone receptors, to investigate how the genes in the ubiquitin-NFκβ respond to changes in oestrogen and progesterone.