**The Institute of Cancer Research**

## PHD STUDENTSHIP PROJECT PROPOSAL:

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| **PROJECT DETAILS** | |
| **Project Title:** | Targeting the activated stroma to limit breast cancer metastasis |
| **SUPERVISORY TEAM** | |
| **Primary Supervisor(s):** | Clare Isacke |
| **Additional members of the supervisory team:** | Amanda Swain |
| **Lead contact person for the project:** | Clare Isacke |
| **DIVISIONAL AFFILIATION** | |
| **Primary Division:** | Breast Cancer Research |
| **Primary Team:** | Molecular Cell Biology |
| **PROJECT PROPOSAL** | |

**BACKGROUND TO THE PROJECT**

Many sold tumours are characterised by a significant infiltration of fibroblasts and a proportion of these will acquire an activated cancer-associated fibroblast (CAF) phenotype. There is now extensive evidence functionally implicating CAFs in tumour progression via their ability to deposit and remodel the extracellular matrix, secrete pro-tumourigenic factors (Kalluri, 2016).

Our laboratory takes a number of approaches to address the mechanisms by which CAFs, and other mesenchymal stroma cells such as pericytes, promote the growth of breast cancers, and their role in promoting metastatic dissemination and colonisation of distant site in the body (Avgustinova *et al.*, 2016; Viski *et al.*, 2016). Of particular interest has been the Endo180 receptor (also known as MRC2, CD280, uPARAP). We have demonstrated that Endo180 is predominantly expressed by CAFs in the tumour microenvironment (with lower level expression in normal tissue fibroblasts), where it functions as a promigratory, novel collagen update receptor (Sturge *et al.*, 2006; Wienke *et al.*, 2003) (Fig. 1).

Our mechanistic studies in 3D in vitro co-culture models have revealed that Endo180 expression on CAFs is required for CAFs to effectively engage and contract matrix components and to infiltrate into the tumour mass. Importantly, we have used the Endo180 knockout mouse that we generated (East *et al.*, 2003), to demonstrate that targeted deletion of the Endo180 receptor has no impact on normal tissue homeostasis but significantly impairs both primary breast cancer tumour growth and metastatic colonisation of the lung, liver and bones.

Together, these data provide pre-clinical evidence that targeting Endo180 may server to limit the pro-tumourigenic features of the tumour microenvironment with limited toxicity to normal tissues.

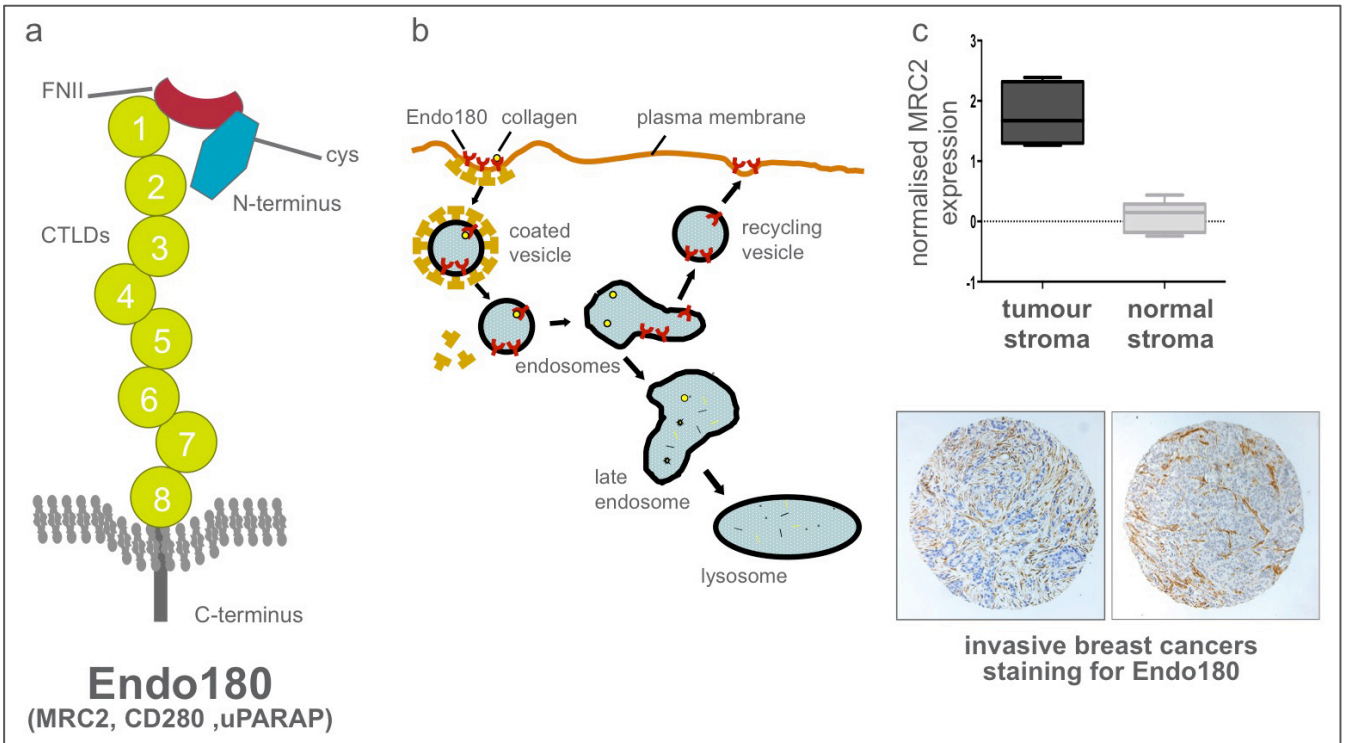


**Fig. 1**. (a) Structure of Endo180, (b) Endo180 is a recycling endocytic receptor that internalises ligands fo degradation in the lysosome. (c) Endo180 expresson is significantly higher in the tumour stroma compared to normal tissue stroma. Immunohistochemical staining of Endo180 in human breast cancers shows that Endo180 expression is restricted to the cancer associated fibroblasts (CAFs).

**PROJECT AIMS**

This overall goal of this project is to develop strategies to target the pro-tumourigenic activated stroma

Using a combination of biochemical, in vitro, ex vivo and in vivo approaches the student will

a. Develop reagents and methodologies for targeting Endo180+ve cells
b. Assess the efficacy of these targeting reagents in both in in vivo/ex vivo metastasis models
c. Further explore the mechanisms by which CAFs promote metastatic dissemination and colonisation.

The project will initially focus on the stromal receptor Endo180 but it is anticipated that the reagents and methodologies developed, together with the experimental approaches to be taken, will integrate closely with other projects in the laboratory to inform more broadly our understanding of the biology of tumour progression in vivo.

**RESEARCH PROPOSAL**

Our laboratory has developed a number of reagents for this project. These include (i) 6 mouse monoclonal antibodies (mAbs) directed against human Endo180, (ii) human and mouse full length and mutated Endo180 cDNA constructs and Endo180-Fc constructs, (iii) Endo180 knockout (Endo180-/-) mice backcrossed onto three different strains including mice with GFP driven by the ubiquitously expressed ubiquitin promoter and by the alpha smooth muscle actin promoter, (iv) syngeneic mouse models of spontaneous metastasis, (v) methodologies for the expression and purification of Endo180 (and other)-Fc constructs, (vi) ex vivo and 3D in vitro co-culture models

**Aim 1: Develop reagents and methodologies for targeting Endo180+ve cells**

We have previously generated and characterised 6 mAbs directed against human Endo180. We are currently generating mAbs directed against mouse Endo180.

The student will, in collaboration with Sophia Karagiannis's group at the Breast Cancer Now Unit at King's College London (Hoffmann *et al.*, 2018), generate a series of Fc-modified and/or antibody drug conjugates (ADCs) based on these mAbs. Using cultured Endo180+/+ and Endo180-/- cells, the student will assess in vitro toxicity of the Fc-modified antibodies/ADCs and optimal therapeutic doses. In addition, as Endo180 is a recycling receptor the student will assess the efficiency of ADC uptake into the cells and the efficiency of cell killing in wild-type Endo180 expressing cells compared to cells expressing an non-internalising Endo180 mutant (Wienke *et al.*, 2003).

**Aim 2: Assess the efficacy of the anti-Endo180 ADCs in in vivo/ex vivo metastasis models**

The student will assess the efficacy of the anti-mouse Endo180 ADCs to target CAFs using the syngeneic BALB/c mouse mammary carcinoma cell lines 4T1 and D2A1, and with the D2A1 metastatic sublines that we have generated (**Jungwirth *et al.*, 2018**). Control naive and tumour-bearing Endo180-/- BALB/c mice will be used to assess off target toxicities. We will assess the following in these experiments;

- ADC half life and distribution
- off tumour and off target toxicities
- evidence of activated stromal cell depletion in both the primary and metastatic sites
- impact on tumour burden, metastatic spread and abundance of circulating tumour cells.

Dependent on the results obtained, these approaches will be extended to human cell lines and patient-derived xenografts (PDXs) implanted into immunocompromised mice and/or to other syngeneic models on a C57BL/6 and/or FVB background. In addition, the student will investigate the optimal timing of treatment and efficacy in combination with conventional (e.g. chemotherapy) or tumour targeting agents.

In parallel the student will test the efficacy of anti-human Endo180 Fc-modified antibodies/ADCs in in vitro CAF-tumour cell co-culture models.

**Aim 3: Further explore the mechanisms by which CAFs promote metastatic dissemination and colonisation**

Our previous studies clearly demonstrate that genetic deletion of Endo180 significantly impairs tumour progression and metastasis and, as a consequence, that targeting Endo180+ve cells in the tumour microenvironment offers a promising therapeutic strategy. However, important biological questions as to the function of Endo180 in CAFs remain. In particular, it is now clear that there is significant heterogeneity in fibroblasts in both normal and tumour tissue (Cortez *et al.*, 2014; Costa *et al.*, 2018). Our preliminary data shows that Endo180 is predominantly expressed in a matrix-producing subset of fibroblasts. The student will further characterise this subset of Endo180+ve CAFs in terms of the

- distribution in normal tissue, the primary tumour microenvironment and metastatic tumour sites
- specific functional properties associated with this CAF subset
- the consequences of targeting Endo180+ve CAFs on other CAF subsets

**Conclusion**

This is an exciting project addressing an important issue in cancer research - "identifying therapeutic strategies for targeting the tumour microenvironment to limit metastatic colonisation" The project is focussed on the stromal receptor Endo180 but it is anticipated that the reagents and methodologies developed, together with the experimental approaches to be taken, will integrate closely with other projects in the laboratory to inform more broadly our understanding of the biology of tumour progression in vivo.

**LITERATURE REFERENCES**

Avgustinova, A., Iravani, M., Robertson, D., Fearns, A., Gao, Q., Klingbeil, P., Hanby, A.M., Speirs, V., Sahai, E., Calvo, F. and **Isacke, C.M.** (2016). Tumour cell-derived Wnt7a recruits and activates fibroblasts to promote tumour aggressiveness. *Nat Commun* **7**, 10305.

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Costa, A., Kieffer, Y., Scholer-Dahirel, A., Pelon, F., Bourachot, B., Cardon, M., Sirven, P., Magagna, I., Fuhrmann, L., Bernard, C., *et al.* (2018). Fibroblast Heterogeneity and Immunosuppressive Environment in Human Breast Cancer. *Cancer Cell* **33**, 463-479 e410.

East, L., McCarthy, A., Wienke, D., Sturge, J., Ashworth, A., and **Isacke, C.M.** (2003). A targeted deletion in the endocytic receptor gene Endo180 results in a defect in collagen uptake. *EMBO Rep* **4**, 710-716.

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Jungwirth, U., van Weverwijk, A., Melake, M.J., Chambers, A.F., Gao, Q., Fivaz, M., and I**sacke, C.M.** (2018). Generation and characterisation of two D2A1 mammary cancer sublines to model spontaneous and experimental metastasis in a syngeneic BALB/c host. *Dis Model Mech* **11**.

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Wienke, D., MacFadyen, J.R., and **Isacke, C.M.** (2003). Identification and characterization of the endocytic transmembrane glycoprotein Endo180 as a novel collagen receptor. *Mol Biol Cell* **14**, 3592-3604.

| **CANDIDATE PROFILE** | |
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| Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1) | |
| **Pre-requisite qualifications of applicants:** | BSc (1st or 2.1) or equivalent, in biological sciences, Biological chemistry or other relevant degree subjects |
| **Intended learning outcomes:**<br>Please provide a bullet point list (maximum of seven) of the knowledge and skills you expect the student to have attained on completion of the project. | (1) A thorough understanding of the biology of cancer metastasis<br>(2) Advanced skills in the development and exploitation of in vitro and in vivo tumour-stroma interactions models<br>(3) Advanced skills in a wide range of molecular, cellular and biochemical assays<br>(4) Ability to design, organise and implement complex experiments<br>(5) Ability to productively liaise with internal and external collaborators<br>(5) Ability to present scientific data both orally and in written form<br>(6) Ability to keep up with, and critically appraise, the relevant scientific literature |