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TITLE: The emerging role of neutrophils in cancer progression

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INTRODUCTION:

Cancer is a highly variable disease. Progression and prognosis are dependent on a number of different factors, which are still being understood. Immune system activation is known to be an important factor influencing cancer outcomes. Many modern cancer treatments modulate the patient's immune cells to heighten the natural immune response against a tumour. The component of the immune system examined during this studentship is the neutrophil, a cell type belonging to the innate immune system. Neutrophils make up 50-70% of the white blood cell population, and in injury and infection, they are the first cells to respond, causing inflammation at injury sites, and recruiting other immune cells. They are highly anti-microbial, attacking micro-organisms by phagocytosis and release of toxic anti-microbial agents from their cytoplasmic granules. In cancer, neutrophils are believed to attack neoplastic cells on first entry into a tumour. With extended exposure to the unique microenvironment of the tumour, the cytotoxic function of the neutrophil is manipulated by factors released by cancer cells, and the neutrophil adopts a new phenotype. These tumour-associated neutrophils encourage invasion and metastasis, and inhibit tumour suppression by the rest of the immune system. Other factors influencing cancer progression and outcome include comorbidities such as obesity, diabetes and other metabolic diseases. These typically lead to a poorer prognosis than in otherwise healthy patients. It is possible that the reason behind these differences in progression is linked to differences in immune infiltrate.

AIM:

The aim of this project was to determine the number, function and location of neutrophil populations in primary and metastatic breast cancer tumours. We aimed to collect data from tissue samples through immunohistochemistry, with a view to using this data to evaluate relationships between tumour-associated neutrophils, comorbidities, invasion and metastasis, and cancer outcome.

METHOD:

I developed immunohistochemical methods to identify and quantify neutrophil populations within mouse breast cancer tumours. The tissues used for this aspect of the project were primary tumours and metastases taken from both wild-type mice and mice with genetic knockouts simulating obesity and metabolic syndrome. Immunohistochemistry uses antibodies developed against certain proteins to detect these proteins within tissues. Due to the specific nature of antibody-antigen binding, the antibodies will only bind to the tissue at locations where the target proteins are present, and these locations can then be visualized using enzymes that catalyse colour-producing reactions. Under a microscope, these colours can be seen and the proteins visualised in the stained tissue. To evaluate the presence and function of neutrophils in the mouse tumours, antibodies against several different proteins were used.

The first antibody, NIMP-R14, is specific for Ly6C and Ly6G cell surface proteins, which are only found on the cell surface of mouse neutrophils, allowing visualization of the neutrophil population. An antibody against myeloperoxidase, which is the main anti-microbial and anti-tumour chemicals produced by neutrophils, identified the cytotoxic properties of the cells. A final antibody, against the CD3 protein, allowed detection of T cell numbers and evaluation of whether the neutrophil population was enhancing or reducing the anti-tumour function of other parts of the immune system. These

target proteins were chosen to provide a basic picture of the neutrophil population's size, location, and function. The principles behind the study of the mouse tumours were then transferred to human tissues, for evaluation of neutrophil populations in microarrays of human breast tumour tissues. The tissue microarray contained core samples from ninety breast cancer patients. Antibodies against CD66b, which is the human equivalent of Ly6C/G, as well as myeloperoxidase and CD3 were used to stain the tissues.

RESULTS:

In mouse tissues, I was able to develop a reliable method for the identification of CD3 proteins within the tumour. However, the control stains for the anti-myeloperoxidase and NIMP-R14, (omission of primary antibody and omission of both primary and secondary antibodies) showed similar staining patterns to the experimental stains. This made it difficult to differentiate true positive results from false positives. In contrast, immunostaining of human breast tumour tissues produced specific and reliable staining for all three protein markers. Data collected from the tissue microarrays is currently being analysed to find relationships between tumour-associated neutrophil location and number and markers of tumour progression, metastasis and adaptive immune response.

Conclusion:

We believe that the false positive results seen in the negative controls in the mouse samples were due to compromise of the visualisation methods required for the anti-myeloperoxidase and NIMP-R14 antibodies by the presence of endogenous enzymes within the tissues. This enzyme activity was elevated in tissues believed to have high neutrophil numbers, to the extent that the standard enzyme blocking methods used within immunohistochemistry were unable to neutralise it. Further development is needed to identify ways to block these endogenous enzymes. The methods developed during this studentship will help direct future studies of tumour immune infiltrates. As neutrophils are so populous within the body, evaluation of their role in tumour progression could have a significant impact on cancer diagnosis, outcome prediction, and treatment methods and could contribute to expansion of our understanding of cancer biology.