**YEAR 2014/15**

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**INSTITUTION: NUI, GALWAY**

**PROJECT TITLE: ANALYSIS OF H2AX PROTEIN ABUNDANCE IN HUMAN BREAST CANCER CELLS**

Brief Resume of your Project's outcomes for the Society's Website: (no more than 200-250 words).

The title of your project and a brief 200-250 word description of the proposed/completed project. The description should include sufficient detail to be of general interest to a broad readership including scientists and non-specialists. Please also try to include 1-2 graphical images (minimum 75dpi). NB: Authors should NOT include sensitive material or data that they do not want disclosed at this time.

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| Analysis of H2AX protein abundance in human breast cancer cells | | | |  |
| DNA in the nucleus of cells is packaged by histone proteins to form chromatin. H2AX is a variant of the core histone H2A and plays a role in the DNA damage response. This is a mechanism that cells use to detect any damage to DNA. DNA damage can lead to mutations and ultimately cause cancer. This project aims to understand more about the role of H2AX in cancer by investigating the amount of this protein in cells which have come from breast cancer compared to normal breast cells. | | | | |
| The following table displays key information on the cells that were used during this project. | | | | |
| Cell Line | Description of cell line | Type | Prevalence (Approx) | Characteristics |
| MCF10A | Non-tumorigenic breast cells |  | n/a | Normal  ER+, PR+, HER2+ |
| MCF-7 | Breast cancer cells | Luminal | 40% of breast cancers are of this type | Patients are endocrine  responsive  Often chemotherapy  responsive  ER+, PR+/", HER2’ |
| MDA-MB-231 | Breast cancer cells | Triple negative | 15-20% of breast cancers are of this type | Patients have intermediate response to chemotherapy Triple Negative ER\ PR', HER2" |
| ER+/- = estrogen receptor-positive/negative  PR+/- = progesterone receptor-positive/negative  HER2+/- = HER2/neu receptor-positive/negative | | | | |
| Two quantitative techniques were used in this project;  1. Fluorescent western blotting  This allowed target histones to be identified and quantified using fluorescent secondary antibodies. Quantification of the amount of H2A and H2AX in each sample was determined by comparison with recombinant standards.  2. 2D gel electrophoresis  This technique involved the migration of polypeptides in two dimensions. In the first dimension, separation was based on charge. The second dimension was a conventional SDS PAGE stained with coomassie. Separation was based on molecular weight. Data obtained is preliminary and is to be reported when the technique is fully optimised. Densitometry will be used to quantify.  This allowed us to compare the abundance of the H2AX histone species with total H2A in different cell lines. Detailed analysis of H2AX abundance in various cell types will shed further light on the role this protein plays in the development and in the changes associated with tumorigenesis. | | | | |

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