Introduction

Synovial Sarcoma (SS) is a malignant soft tissue tumour. It accounts for 10% of all soft tissue sarcomas. It is genetically characterised by the chromosomal translocation t(X;18)(p11;q11) which leads to the fusion of the SS18 (SYT) gene to SSX1, SSX2 or SSX4 (Trautmann et al, 2013).

This translocation is demonstrated in almost all cases of SS and is specific for this sarcoma. The expression of the SS18/SSX is both necessary and sufficient for the oncogenic activity of SS and for tumorigenesis (Su et al, 2012; Rota et al, 2012). The control of gene expression by SS18/SSX involves chromatin remodelling due to interactions with polycomb group (PcG) proteins.

Currently there is no drug on the market that specifically targets the fusion protein or mutation. This study attempted to identify such a drug.

Aims

The aims for this project were to:

- Analyse the interaction of SS18/SSX oncogene with the polycomb group protein TLE-1.
- Evaluate the effect of specific drugs (Menadione, B2 and TV-6) on this complex and to analyse if they break this interaction.
- These were determined with Proximity Ligation Assay (PLA) and Immunoprecipitation (IP) with Western Blot (WB).

Methods

The following details the methods employed in this study;

- Perform Antibody characterization via immunofluorescence for Proximity Ligation Assay (PLA).
- Analyse interactions between proteins via PLA.
- Protein interactions were also confirmed via Western blot and immunoprecipitation.
- Drug inhibition of SS will be evaluated via PLA and also IP

Results

The H80 antibody recognises the SS18 protein. It also recognises the fusion protein SS18/SSX. In K-SS1 the antibody gave cytoplasmic staining whereas in Mojo it gave both cytoplasmic and nuclear staining. The control band is not visible as an SSX control was used and H80 recognises only SS18.

We used H80 antibody and TLE1 antibody and both these antibodies together should recognise the fusion protein SS18/SSX. We analysed the interaction of these proteins and visualised the location of these interactions. H80 and TLE shows very nice PLA signals. The interaction is both nuclear and cytoplasmic but it is mainly nuclear. This suggests that TLE protein is interacting with either the fusion gene or SS18.

Figure 1: SS18 expression using H80 antibody in A) Western Blot and B) Immunofluorescence

Figure 2: PLA with Syo-1 cell line using antibodies at a 1:500 concentration

Discussion and Conclusions

Syo-1 shows strong nuclear fluorescence therefore it was selected for further analysis based on these results. It was decided to use H80 antibody for PLA and IP based on the WB and IF. This antibody has been shown to recognise not only SS18 but also the fusion protein SS18/SSX evident in figure 1.

This study looked at the interactions between the fusion protein and TLE-1. The results showed that there was a positive interaction between these two proteins (figure 2). It was our aim to analyse if this interaction could be broken by current drugs active on SS.

Based on the results in figure 3, the PLA count was as expected from treated cells. Based on the number of PLA signals detected, the treatments decreased the H80-TLE1 interactions slightly. It is evident from the graph that Menadione has a slight improvement on the treatment of SS over the other two drugs, Tenvin 6 and compound B2.

It can be concluded from this study that there are drugs on the market such as the ones used in this research that may be targeted towards breaking this protein interaction and this looks promising for future therapy of SS.

References


Author’s Email: avrilmadden22@gmail.com