**The Ubiquitin Proteasome System and Cancer**

The ubiquitin proteasome system is a central component of the cellular degradation pathway involved in cellular growth control. Therapies targeting the proteasome, the endproduct of this pathway, are now used for the treatment of cancers. This chapter focuses on the commercialization of protein degradation enzymes, with particular emphasis on E6AP ligase and RING E3 ligase activity.

**ESAP As a Drug Target**

ESAP is a HECT domain E3 ligase critical for the suppression of p53 in human papilloma virus (HPV)-related cancers, and is a target for drug development. The ubiquitination of p53 by ESAP is critical to tumourigenesis. Small molecule inhibitors of ESAP could therefore provide a novel therapeutic strategy for cancer treatment.

**Protein Production**

For the production of ubiquitin, we used the Alphascreen technology to screen our library of 250,000 compounds. In addition, we applied this technology to screen for ESAP ligases.

**Hit Triage Process**

Two independent HTS campaigns were undertaken using our ESAP primary screen. The first campaign was directed against ESAP (HECT domain E3 ligase), and the second campaign against a RING domain E3 ligase. By performing two independent HTS campaigns, we were able to validate our ability to identify compounds that interfere with the common components of both assays (E1 ligase, E2 ligase and Alphascreen technology).

**Conclusions**

A robust assay platform has been established for the detection of both alpha- and beta-subunit ubiquitination utilizing Alphascreen technology. This assay was used to screen a library of 250,000 compounds against the E3 ligase ESAP and a RING domain E3 ligase also implicated in cancer.

**References**

1. Losch-Hayes, E. et al., Oncogene 2012 Apr 23;31(17):2199-2209