Detection and Quantification of Copper Catalyzed Bio-Orthogonal Fluorescent Labelling of Lewis Lung Carcinoma Cells

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Labelling proteins with antibodies or bulky functional groups interferes with normal structure, while radioisotope labelling creates environmental hazards. Click chemical reactions advantageously preserve biological structure and function without creating toxic byproducts. The copper catalyzed click formation of a triazole between an azide and alkyne groups results in a stable bond. Azide and alkyne functional groups have potential application towards cellular labelling because these groups rarely occur naturally. Previously we successfully labeled cells lacking the gene TSC2 (tuberous sclerosis complex) responsible for regulating growth and proliferation. This demonstrates that a fast growing cell line has the capacity to be labelled. To broaden the application of this technique, we extended the protocol to Lewis lung carcinoma cells , a cell line that does not display growth dysregulation. Azidohomoalanine (aha), a methinonine analog with azide functionality, was used to label proteins. To optimize cell labeling, we varied the length of aha incubation, doses of aha, and washing methods, and length of aha-free media pulse. Cells were prepared for flow cytometry by paraformaldehyde fixation overnight followed by labelling with Alexa Fluor 488-alkyne in a click reaction mixture. Doses of aha were tested on a logarithmic scale. 1µM dose of aha was selected as doses above 1µM did not confer a significant increase in fluorescence intensity or percentage of cells labeled. The optimal incubation time for maximum aha incorporation of a 1µM lies between 9 and 24 hours. The optimal number of washes was determined to be 2 which streamlined the overall labeling protocol. These studies have established that we can use aha to label carcinoma cells and detect that label using click chemistry. This labeling technique produces stably labeled cells that can be tracked for up to three generations and used to examine tumor growth in vivo.