2014 Joint Usage and Research Report

Title of Research Project		Analysis of the molecular mechanisms underlying the mutual interactions between normal cells and transformed cells in epithelia in vitro and in vivo
Applicant	Institution	University College London
	Job title and name	Dr. Masazumi Tada
Visiting researcher	Name	Dr. Katarzyna Anton
Purpose of the Research Project (approx. 250 words)		The aim of this project is to investigate mutual interactions at the interface between normal cells and transformed cells at the initiation of carcinogenesis in the two different model systems: Madin_Darby Canine Kidney (MDCK) cells and the zebrafish embryonic epithelium. Fujita and Tada labs found out the conserved mechanisms, by which transformed cells send signal(s) to neighboring normal cells and in turn the normal cells respond to the transformed cells, resulting in transformed cell extrusion from the epithelium. The initial research purpose upon this grant application was to uncover novel mechanisms underlying transformed cell extrusion by comparing the differences in outputs between the two model systems. However, we decided to refocus to further identify the novel conserved mechanisms, since our collaborative paper (Kajita et al., 2014) was published in Nature Communications. Tada lab found out a potentially novel mechanism in zebrafish but impinged upon difficulties to demonstrate this based on in vivo time-lapse analysis because of the limited availability of reagents. To complement this, Fujita lab offered to use MDCK cells to override the difficulties. Based on several lines of experimental evidence, we hypothesized that transformed cell extrusion is regulated by cell cycle progression. To test this hypothesis, Dr Anton from Tada lab visited Fujita lab, and carried out experiments in MDCK cells using the reagent that allows us to visualize cell cycle transition in live imaging. (227 words)

Development of the Research Project and Results (approx.. 850 words)	Real time imaging analysis of transformed Src cell extrusion in the zebrafish embryonic epithelium revealed that an extruding Src cell is highly associated with the presence of a small cell or part of the cell on the opposite side of the extruding Src cell from the epithelium. Importantly, this cell-like structure rarely contains the nucleus and aster microtubules. This is not in an authentic manner but reminiscent of cell division in the epithelial plane perpendicular to the orthogonal axis, as opposed to normal cells divide in the same plane. Thus, Src cells might be extruded from the epithelium through atypical or asymmetric cell division. This prompted us to test the hypothesis that Src cells might be extruded in the timing of cell division from the host epithelium. To support this hypothesis, we demonstrated that inhibition of G2/M transition, but not of G1/S transition, effectively suppressed Src cell extrusion from the zebrafish epithelium. However, it was technically challenging to capture images of extruding Src cells in the phase of cell cycle (G1, S or G2) in the embryonic epithelium, due to rapid cell cycle progression, although we tested several different reagents in zebrafish. During her visit to Fujita lab, Dr Anton further investigated the relationship between the cell cycle progression and Src cell extrusion in MDCK cells. To consolidate this idea, first Dr Anton was able to inhibit Src cell extrusion using an inhibitor specific for G2/M transition, but not for G1/S transition. Along with Dr Kajita in Fujita lab, next Dr Anton has established a stable MDCK cell line harboring the live imaging marker for cell cycle transition called Fucci in which we can visualize changes in colors in the different phases (red in G1 and green in S, G2 or M). This fluorescent reporter line allowed us to examine if the cell cycle progression occurs in relation to Src cell extrusion. The time-lapse analysis using the Fucci reporter line revealed that Src cells become extruded predominantly in the phase, G2/M transition. Finally, using the FACS cell sorter Dr Anton assessed if DNA contents in both normal and transformed cells are correlated with their state in cell cycle progression. Because of the limited time (for only 5 weeks), Dr Anton could not have a conclusive result from the last experiment. Together with the data in zebrafish, Dr Anton demonstrated that the timing of Src

	cell extrusion is highly correlated with the cell cycle progression, in particular G2/M transition. This collaborative project enabled us to validate our hypothesis in the two different systems in vitro and in vivo, and the data from this work will be contributed to an important part of publication in the near future. Currently, we are planning to prepare a manuscript aiming at a highly profiled journal, provisionally entitled "G2/M transition is a morphogenetic check-point that determines elimination of transformed cells from host epithelia". Other than the main part of the achievement by Dr Anton, during my visit (for 5 days), Fujita and Tada labs both exchanged the data and idea in several on-going projects. Of those, a project related to transformed cell extrusion and autophagy was particularly interesting to both of us. Dr Kon in Fujita lab found that the molecular/genetic pathways activating autophagy are involved in the regulation of transformed cell extrusion in MDCK cells. On the other hand, Tada lab has experimental evidence that an oncogenic form of the tumor suppressor gene Adenopolyposis coli (Apc), which has been found in 80% of colorectal cancer cases in humans, is capable of activating the autophagy pathway during cell extrusion and acts independently of canonical Wnt signaling. Despite the fact that the extrusion rate of Apc cells is much lower than that of Src cells, that of Apc cells is much more facilitated in other genetic backgrounds. This increased extrusion rate is highly correlated with more aggressive behavior of Apc cells than in wild type background. These data prompted us to raise the hypothesis that the molecular/genetic pathways upstream of autophagy might play a pivotal role in the regulation of transformed cell extrusion in particular in the Apc mutant background. We will explore this project further in the two systems, in addition to each project in either lab. Thus, we will aim to uncover a new aspect of cancer progression in a multiple-hit model using the two model systems: in vitro and in vivo.

	Overall, this joint grant has led us to fruitful collaborations, through which we will be able to publish several good papers in the coming year or two. We would like to keep this collaboration in a longer term, so that we are planning to apply for another collaboration grants such as one from the Royal Society, UK. (784 words)
Publication *Enter the names of conference or journal and its vol. No. where the above work was presented.	【Conference, symposium, workshop etc.】 Not yet.
	【Journals】 Not yet.