Centrosome amplification and Signaling Transduction

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***Short Abstract* — Normal and tumor cells often differ in their number of centrosomes: normal cells contain two centrosomes during mitosis, whereas tumor cells often contain >2 (referred to as centrosome amplification). Centrosomes are required to organizing the poles of the mitotic spindle and the equal segregation of the genetic material into two daughter cells, but they also have important functions during interphase. Our data show that amplification of centrosomes and increased microtubules nucleation induce increased Rac Activity, alter phosphotyrosin localization at focal adhesions, and promote enhanced cellular motility and invasive ability. These results suggest that centrosome amplification could potentially alter cell anchorage, motility and transmembrane signaling.**

I. Purpose

Centrosome amplification is a characteristic of many solid tumors where it correlates with early stage (pre-invasive) and tumor malignancy [1-4]. Although centrosomes are primarily involved in organizing the poles of the mitotic spindle, as required for the equal segregation of the genetic material into two daughter cells, they also have important functions during interphase [5]. The requirement for normal centrosome function outside of chromosome segregation is illustrated by the effect of centrosome amplification on asymmetric neuroblast cell divisions in Drosophila [6].

My work builds on the striking results that centrosome amplification induces cell invasion and the formation of unstable cell-cell contacts in a 3D spheroid culture model of human mammary glands (manuscript recently submitted for publication). Furthermore, we have found that cells with centrosome amplification have clustered centrosomes and increased microtubule (MT) nucleation. MT dynamics are known to influence Rho and Rac activity: disassembly of MTs increases Rho and decreases Rac activation, whereas MT re-growth has the reciprocal effect [7-9]. The mechanism underlying this regulation is poorly understood, but requires GEFs [10]. Our results show that cells with extra-centrosomes have an over-activation of Rac, which depends on microtubules dynamics. To measure Rac activation I used a Rac FRET Biosensor and developed an automated imaging analysis system. To investigate whether Rac activity is responsible for the observed cell protrusive activity and the cell-cell adhesion instability in cell with centrosome amplification I use a combination of micropatterning system and Rac FRET biosensor. The advantage of using the micropatterning is the high reproducibility of the cell morphology and cell-cell contact geometry, and, the possibility of acquiring high spatial and temporal resolution of MT dynamics, RhoGTPase activation, and the cell cortex. Micropatterning are produced in-house, by applying my novel technique that is based on plasma surface removal (manuscript recently submitted for publication).

An alternative mechanism that we are also exploring is that amplification of centrosomes and MT nucleation could induce increased turnover of focal adhesions, which could promote enhanced cellular motility and invasive ability [11-13]. Indeed, our data show that cells with centrosome amplification have altered phosphotyrosin localization at focal adhesion. This suggests that centrosome amplification could potentially alter both cell anchorage and transmembrane signaling [14].

Future work will be focused on quantifying the interactions between MTs, RhoGTPases, cell adhesion and the signal transduction componentes (e.g., ERK1/2, JNK, GSK3β) in single cells and populations of cells with centrosome amplification.

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