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Abstract

Important pathological hallmarks of many breast cancers include centrosome amplification, spindle pole defects leading to aberrant chromosome segregation, altered nucleoskeletal proteins and perturbed cytokinesis. Our recent data showing that protein 4.1 is crucial for proper centrosome, spindle and nuclear assembly /maintenance led us to hypothesize that protein 4.1 is involved in centrosome dynamics, fidelity of cell division and cell cycle progression.

To decipher 4.1 functions in breast cancer, we studied the detailed distribution of two 4.1 family members, 4.1R and 4.1G and found that protein 4.1 R and 4.1G localize differentially within centrosomes and spindles and behave differently during the cell cycle. In several breast cancer cell lines with normal centrosome numbers, we determined that 4.1R localizes to one or both centrioles in randomly growing populations. By contrast, in other breast cancer cell lines with amplified centrosomes we detected 4.1R at a subset of hyperamplified centrioles. We are currently investigating 4.1distribution in other breast cancer cell lines and assessing changes after specific 4.1 downregulation by RNAi. Our ultimate aim is to determine if 4.1 or its binding partners could be important chemotherapeutic targets in breast cancer treatment.

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Important pathological hallmarks of many breast cancers include centrosome amplification, spindle pole defects leading to aberrant chromosome segregation, altered nucleoskeletal proteins and perturbed cytokinesis. Previously we established in human cells that protein 4.1, initially described as a human red cell skeletal protein, is also a component of centrosomes, mitotic spindles, nuclear skeleton and the midbody at cytokinesis. Importantly we recently showed by depletion/add back experiments that protein 4.1 itself is crucial for proper centrosome, spindle and nuclear assembly and that dominant/negative 4.1 peptides added to cell extracts produced multipolar and asymmetric mitotic spindles and disrupted microtubule organization necessary to assemble and maintain centrosomes. Taken together, these data lead us to hypothesize that protein 4.1 is involved in centrosome dynamics, the fidelity of cell division and in cell cycle progression.

Protein 4.1 is now known to be a multigene family. We are investigating roles of two family members, 4.1R (red cell) and 4.1G (generally expressed) in centrosome amplification and mitotic spindle aberrations in breast cancer pathogenesis. Centrosomes are composed of a cylindrical centriole pair surrounded by a larger fibrogranular area, the pericentriolar material (PCM). The more mature (or mother) centriole has "appendages" at its distal end that appear to anchor cytoplasmic microtubules. Centrosomes duplicate to become mitotic spindle poles for partitioning chromosomes.

In order to decipher 4.1 functions in breast cancer, we studied the detailed distribution of 4.1R and 4.1G at centrosomes. We report here that, by immunofluorescent microscopy, protein 4.1 R and 4.1G localize differentially within centrosomes. Protein 4.1R surrounds the centriolar "barrel" while 4.1G localizes in the outlying PCM. In mitotic spindles 4.1R is again associated with centrioles while 4.1G is distributed in the spindle matrix. Moreover, we observed that 4.1R is specifically associated with the mature (or mother) centriole, coincident with mature centriole marker proteins ninein and p150/glued by immunofluorescent microscopy. During cell cycling, 4.1R is at one centriole but spreads to the maturing daughter centriole during S phase, finally localizing during G2 at both mature centrioles after centriole duplication. Protein 4.1G epitopes remain in the PCM throughout the cell cycle. Applying these observations to breast cancer cell lines, we find that in breast cancer cell lines with normal centrosome numbers (MCF10A transformed cells and MCF7 tumor cells), protein 4.1R localizes to one or both centrioles in randomly growing populations. By contrast, in breast cancer cell lines with amplified centrosomes, such as MDAMB231and T47D, 4.1R is observed at a subset of hyperamplified centrioles defined by centrin immunostaining. We are currently investigating 4.1R and 4.1G distribution in other breast cancer cell lines in preparation to assess changes in cellular phenotypes after specific downregulation of protein 4.1R and G. Our ultimate aim is to determine if 4.1 or its binding partners could be important chemotherapeutic targets in breast cancer treatment.