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Oncogenic β-catenin mutations evade pH-regulated degradation

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ABSTRACT

β-catenin has roles in cell-cell adhesion and Wnt signaling. We recently showed that β-catenin protein abundance is decreased at higher intracellular pH (pHi), mediated by pH-sensitive interaction with the beta-transducin repeat containing E3 ubiquitin protein ligase (β-TrCP). Increased pHi facilitates β-TrCP binding and degradation of β-catenin. β-catenin mutations that abrogate the pH-sensitive interaction induce significant tumors not seen with other β-catenin stabilizing mutants.

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Transient increases in intracellular pH (pHi) have been shown to be either necessary or sufficient for diverse cellular processes such as directed cell migration,[1] cell cycle progression,[2] and differentiation.[3] Dysregulated pHi dynamics are a hallmark of diseases such as cancer, where increased pHi enables various cancer cell behaviors.[4,5] These cell behaviors are mediated by proteins termed pH sensors whose activity, binding, or localization are sensitive to physiological changes in pHi.[6] We recently reported that CTTNB1 (catenin beta 1, best known as β-catenin) is a pH sensor, with decreased stability at increased pHi.[7] At high pHi, β-catenin association with beta-transducin repeat containing E3 protein ligase (β-TrCP) is increased, leading to lower levels of β-catenin at junctions, in the nucleus, and in whole cell lysates. We identified a single histidine (His) residue in β-catenin that mediates this pH sensitive function. When that evolutionarily-conserved histidine (His36 in Human β-catenin) is mutated to a non-titratable residue, pH-sensitive binding to β-TrCP is abrogated. Moreover, this His is mutated in human cancers to arginine (Arg), and when we expressed the analogous mutation (His42Arg) in *Drosophila* eyes, we observed Wnt signaling activation as well as formation of ectopic tumors not seen with other stabilized β-catenin mutants. Our results suggest that pHi dynamics regulate Wnt signaling by modulating β-catenin stability, and that cancer-associated mutations circumvent physiological mechanisms that decrease Wnt signaling at increased pHi.

After determining that increased pHi produced a strong dysplasia phenotype in the *Drosophila* eye,[8] we performed a genetic screen to identify pH sensitive proteins. We identified *armadillo* (*arm, Drosophila* β-catenin) in this screen and found that overexpression of *arm* suppressed the dysplasia phenotype. We confirmed that Arm was decreased at cell-cell junctions in the *Drosophila* eye as well as in whole head lysates with increased pHi. Importantly, we saw no change in total levels of other adherens junction proteins such as Shotgun (*Drosophila* E-cadherin) or N-cadherin. We confirmed these results in mammalian epithelial cells, and demonstrated that both junctional and nuclear β-catenin levels were decreased at higher pHi. These data suggested that increased pHi was leading to decreased β-catenin abundance, and we confirmed that β-catenin degradation was increased at high pHi using a metabolic pulse-chase assay.

β-catenin levels are regulated primarily by ubiquitination and proteasome-mediated degradation. Degradation requires obligate phosphorylation of N-terminal residues of β-catenin by the kinases casein kinase 1 (CK1) and glycogen synthase kinase-3 beta (GSK3-β) for recognition by the E3-ligase β-TrCP. We first tested whether high pHi was increasing phosphorylation of β-catenin by these kinases, leading to increased degradation at high pHi. However, we found no difference in phosphorylation of β-catenin by these kinases either *in vitro* or in cells. Our next hypothesis was that β-catenin binding to the E3-ligase β-TrCP was sensitive to changes in pH. β-TrCP binds to an evolutionarily-conserved destruction motif (DSGIHS) in β-catenin (Figure 1). Since histidines can titrate within the physiological range and can function as molecular switches, we predicted that pH-sensitive binding of β-catenin to β-TrCP might be mediated by the conserved histidine residue in the destruction motif of β-catenin. Based on the published crystal structure of β-catenin complexed with β-TrCP,[9] we predict that at lower pHi, a protonated histidine will repel the positively charged Lysine 365 on β-TrCP to reduce binding (Figure 1, left panel). At increased pHi, we predict that deprotonation of this histidine will promote binding to β-TrCP, leading to the observed decreased in total protein levels (Figure 1, center panel). Consistent with this model, we

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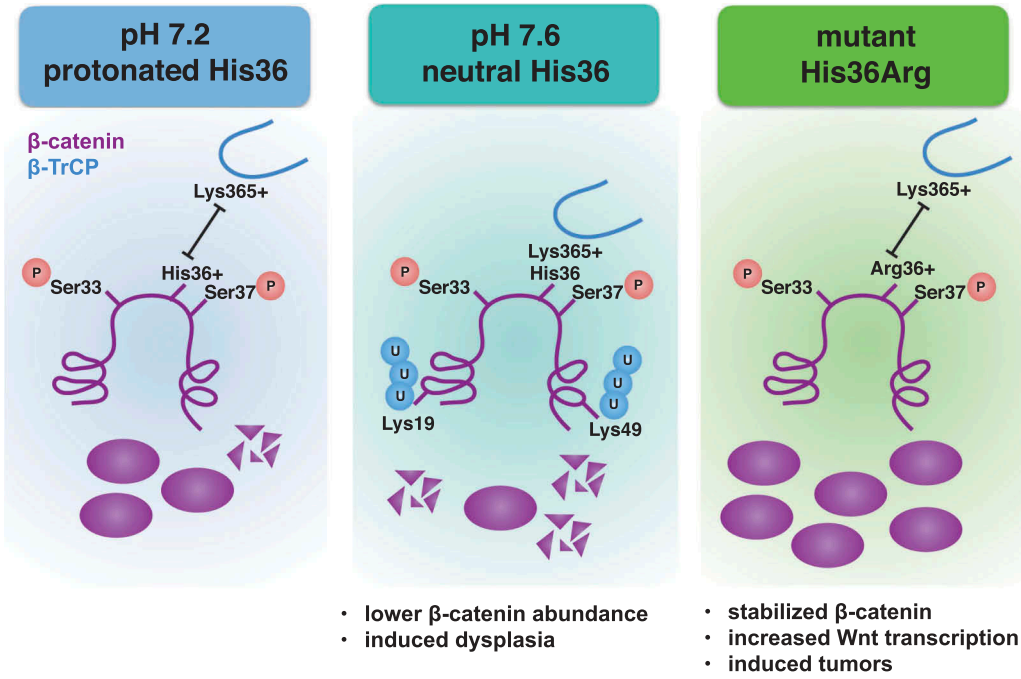

Figure 1. Potential pH-sensing mechanism of β-catenin. Obligate phosphorylation is unchanged with changes in intracellular pH (pHi), but titration of a single histidine residue with changes in pHi is sufficient to alter binding of β-catenin (purple) to beta-transducin repeat containing E3 protein ligase (β-TrCP, blue). We predict interactions between His36 and Lys365 in β-TrCP mediate the pH sensitive binding. At high pHi, β-catenin interaction with β-TrCP is increased, leading to decreased protein abundance and dysplasia. When the cancer-associated arginine mutation is present, pH sensing is abrogated, β-catenin is stabilized, Wnt signaling is increased, and ectopic tumors are formed in the fly eye.

report that β-catenin binds to β-TrCP with higher affinity at high pH. Supporting our hypothesis, when we mutated that histidine residue to a non-titratable arginine (His36Arg, Figure 1, right panel) or alanine residue, we lost pH sensitive binding.

After determining that His36 is the residue mediating pH-sensitive β-catenin abundance, we asked whether this residue is mutated in disease. We found that His36Arg is a recurrent mutation in the Catalog of Somatic Mutations in Cancer, associated with liver and biliary tract tumors (COSMIC Mutation ID: COSM27378). When we mutated the analogous residue (His42Arg-Armadillo) and expressed it in the developing *Drosophila* eye, we observed increased Wnt signaling resulting in large, protruding tumors. This phenotype is very different than the *Drosophila* eye phenotype that results from other stabilized β-catenin mutants, which produce reduced, rough eyes and no protruding tumors. These data are, to our knowledge, the first studies of this cancer-associated mutation, and support a mechanism whereby His36Arg increases transcription of Wnt target genes and uniquely has tumorigenic phenotypes that are not seen with other β-catenin mutations that alter phosphorylation or ubiquitination.

Our work introduces a novel effect on β-catenin whereby increased pHi functions in coincidence with obligate phosphorylation to regulate proteasome-mediated β-catenin degradation. Given that increased pHi is an early event in cancer development,[10] we predict that pH-sensitive loss of β-catenin from cell-cell junctions may be one mechanism mediating dysplasia initiation and early metastasis. Additionally, mutations that stabilize β-catenin protein by altering phosphorylation or ubiquitination may show stabilized β-catenin levels even at the higher pHi found in cancer cells, bypassing this pH-regulatory step and elevating Wnt signaling, which is associated with breast, colon, and liver carcinomas.[11]

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