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Role of Calcium sensing receptor (CaSR) in tumorigenesis

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The extracellular Ca^{2+}-sensing receptor (CaSR) is a robust promoter of differentiation in colonic epithelial cells and functions as a tumor suppressor in colon cancer. CaSR mediates its biologic effects through diverse mechanisms. Loss of CaSR expression activates a myriad of stem cell-like molecular features that drive and sustain the malignant and drug-resistant phenotypes of colon cancer. This CaSR-null phenotype, however, is not irreversible and induction of CaSR expression in CaSR-null cells promotes cell death mechanisms and restores drug sensitivity. The CaSR also functions as a tumor suppressor in breast cancer and promotes cellular sensitivity to cytotoxic drugs. BRCA1 and CaSR functions intersect in breast cancer cells, and CaSR activation can rescue breast cancer cells from the deleterious effect of BRCA1 mutations.

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CaSR expression in the colon and its loss in colonic tumorigenesis and progression

In the normal scheme of colon physiology, rapidly proliferating stem cells at the base of a colonic crypt migrate upwards toward the crypt apex in the direction of the lumen where they assume an increasingly more differentiated phenotype.[1–5] As these cells differentiate, proliferation slows and they become increasingly susceptible to apoptosis. Finally, fully differentiated epithelial cells at the apex of the crypt undergo apoptosis and are extruded into the lumen. This process of migration, differentiation and extrusion takes place over a period of about 6 days.[3,5] Immunohistochemical analysis of histological sections of human colon specimens shows that CaSR expression is restricted to epithelial cells in the colonic crypts.[6,7] Rapidly proliferating stem cells at the base of a crypt, however, do not express CaSR.[6,7] As the crypt stem cells migrate toward the apex they differentiate, proliferation slows and the CaSR is expressed. A relatively high level of CaSR expression is found in cells at the apex where they are non-proliferating and fully differentiated, and ready to undergo apoptosis.[6,7] Thus, CaSR expression is tightly linked to differentiation, reduced proliferation and, ultimately, cell loss in the colonic crypts.

Histological sections of colon carcinomas, on the other hand, show a different pattern of CaSR expression. Differentiated colon carcinomas (retaining glandular or crypt structures) express relatively low levels of CaSR, whereas CaSR expression is lost in undifferentiated carcinomas (with no glandular or crypt structures) and in the invasive front of a differentiated carcinoma.[6,7] Thus, the loss of CaSR expression is linked to tumor formation and malignant progression. Because malignant transformation of colonic mucosa is attributable to a failure of terminal differentiation resulting in regional expansion of proliferating cells,[3,5] loss of CaSR expression, and thus CaSR-dependent control, may allow colonic epithelial cells to diverge from the normal differentiation pathway into a pathway of tumorigenesis and progression.

CaSR function/loss of function in colon cancer

It is well-established that Ca^{2+} possesses chemopreventive properties in colon cancer, i.e., Ca^{2+} acts to suppress or delay colon carcinogenesis.[8–12] Its mechanisms of chemopreventive action, however, are not well understood. Kallay and colleagues reported that the Caco-2 human adenocarcinoma cell line express CaSR, which mediates extracellular $Ca^{2+}{}_o$-dependent inhibition of Caco-2 cell proliferation.[13,14] These investigators further proposed that CaSR could play an important role in the chemopreventive action of Ca^{2+}.

Analyses of a bank of human colon carcinoma cell lines by Western blotting and RT-PCR showed that human colon carcinoma cells express CaSR at both the mRNA and protein levels.[7,15–18] *In vitro* studies using several of these cell lines showed that activation of the CaSR by $Ca^{2+}{}_o$ or other agonists suppresses the malignant phenotype through pathways that inhibit growth and promote differentiation. Activation of CaSR also inhibits invasion and anchorage-independent growth of human colon carcinoma cells.[6,7,15,16] At the molecular level, it promotes expression of the tumor suppressor E-cadherin through the mitogen-activated protein kinase signal pathway,[7,19] up-regulates expression of the growth-inhibitory and differentiation-related $p21/^{Waf1}$ cell cycle check point protein, and expression of γ-catenin and $p27/^{Kip1}$, and suppresses the malignancy-associated β-catenin/Wnt, c-myc and cyclin D1 pathways[6,7,19,20] and suppresses the expression of thymidylate synthase and survivin.[15–17] Some of these pathways have been validated by immunohistochemical analyses of histological sections of human colon.

In normal tissues where CaSR is expressed, β-catenin is expressed at borders between cells.[20] Wnt/β-catenin signaling is considered a major driving mechanism for malignant progression in epithelial cancers[21] and transcriptional activation of β-catenin occurs at the invasive front of colorectal tumor metastases in animal models.[6,21] Consistent with these observations, in tumors with markedly reduced CaSR expression, β-catenin is expressed at high levels in the cytoplasm and nucleus suggesting activation of the β-catenin/wnt pathway.[20] In addition, targeted deletion of the CaSR in the mouse intestinal epithelium in mice was associated with hyperplasia and activated β-catenin signaling.[21]

CaSR modulates the cytotoxic response of colon carcinoma cells to anticancer drugs

Activation of the CaSR inhibits the expression of thymidylate synthase and survivin in human colon carcinoma cells.[15–17] Thymidylate synthase is a key enzyme involved in the de novo synthesis of DNA.