Neuroendocrine Differentiation and Epithelial to Mesenchymal Transition in Prostate Cancer: cAMP-Dependent Signaling as a Therapeutic Target

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Abstract: Prostate cancer exhibits both epithelial to mesenchymal transition and neuroendocrine differentiation. The major barrier to targeting epithelial to mesenchymal transition is that it is heavily involved with normal biology, such as wound repair. In prostate cancer, cAMP can trigger both neuroendocrine differentiation and epithelial to mesenchymal transition in a Snail-dependent manner We will review inhibition of cAMP-signaling as a target for drug development with the goal of simultaneously blocking both neuroendocrine differentiation and epithelial to mesenchymal transition in a tissue and tumor selective manner.

Keywords: Androgen-independent prostate cancer, protein kinase A, adenylyl cyclase, Snail, cyclic nucleotide phosphodiesterase, heterotrimeric G protein.

Prostate cancer is the second cause of cancer mortality among men. Since the 1941 report by Charles Huggins of the therapeutic impact of androgen withdrawal on prostate cancer, medical or surgical castration has been central to the treatment of metastatic prostate cancer. The response rate to this treatment is initially very high and in large randomized trials clinical complete remissions have been reported in close to 15% of men with bone metastases [1]. Despite the initial effectiveness of this treatment, resistance develops in nearly all patients and metastatic prostate cancer is uniformly lethal. This pattern of early responsiveness to treatment followed by evolution of resistance and death is seen in most other cancers when metastatic.

This common and unfortunate outcome stands in marked contrast to several other metastatic cancers that also have a high initial response rate. Hodgkin's disease, some nonHodgkin's lymphoma, acute lymphoblastic leukemia in children, testicular carcinoma and gestational choriocarcinoma can all be cured in a portion of patients with metastatic disease.

One of the enduring puzzles of cancer treatment is why some cancers are curable and others are not. While multiple hypotheses have been proposed, these for the most part fall into two major groups. One line of research suggests that initial therapy fails because cancers exhibit rapid genetic change and at diagnosis pose a wide-range of genetic alterations. Treatment then serves to select for cancer cells ever more resistant to available therapeutic agents until clinical effectiveness is lost.

A second line of investigation suggests that treatment success is limited by the ability of lethal cancers to shift to a state that limits treatment effectiveness. The epithelial – mesenchymal transition (EMT) is one example of this approach that has seen considerable success in explaining some the limits of current cancer treatment.

Epithelial to mesenchymal transition and the reverse process, mesenchymal to epithelial transition (MET) appear to play critical roles in cancer biology. The transition of carcinomas to a mesenchymal phenotype leads to suppression of expression of adhesion proteins. This in turn allows the carcinoma cells to become invasive and metastatic. Once at a metastatic site, transition from the mesenchymal phenotype back to an epithelial phenotype has been proposed to facilitate proliferation. This process makes an attractive therapeutic target as it offers the promise of blocking the invasion and metastatic spread.

One major problem with EMT as a therapeutic target is that this process is also involved in a range of key normal physiologic processes. For example, EMT appears to be central to wound healing. A treatment that blocked wound repair would be potentially catastrophic for a cancer patient as they are often subjected to treatments such as surgery or radiation therapy where healing of normal issue injury is critical.

The blockade of normal physiologic processes during cancer treatment is not the death knell for a

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therapeutic modality. Many of the most dramatic success in cancer treatment were the result of attaining a careful balance between cancer cell kill and normal tissue injury. A majority of the first generation cancer drugs targeted cell proliferation. These agents were consistently toxic rapidly proliferating normal tissues, such as the bone marrow or the lining of the gastrointestinal tract. Nevertheless, these drugs lead to the cure of a range of malignancies, including acute lymphoblastic leukemia in children, Hodgkin's disease, several types of nonHodgkin's lymphoma, gestational choriocarcinoma and carcinoma of the testes. In the process, medical oncologists developed techniques to limit the morbidity and mortality associated with the damage to normal rapidly proliferating tissue.

In this article, we will review some possible options for managing EMT in prostate cancer while limiting damage to normal tissue.

One of the unusual aspects of prostate cancer is that in addition to EMT, prostate cancers can also undergo neuroendocrine differentiation [2]. This phenomenon has been extensively studied in the clinic and there are well-characterized laboratory models [3]. In the process, the prostate cancer cells develop neurite-like extensions and produce secreted proteins like chromogranin A and neuron specific enolase. They also release a range of products able to function in an autocrine or paracrine fashion. While neuroendocrine differentiation is associated with a loss in proliferation, they are able to stimulate proliferation of surrounding prostate adenocarcinoma cells. The latter has been observed in both the clinic and in laboratory models [4].

At first glance, it would appear that EMT and neuroendocrine transformation share little in common. EMT is characterized by loss of adhesion and the acquisition of motility, invasiveness and the capacity for metastatic spread. In contrast, neuroendocrine differentiation is characterized by many of the aspects you would expect to find in any differentiated neuroendocrine cell. However, despite these differences, in prostate cancer EMT and neuroendocrine differentiation are triggered by same external stimuli and are associated with activation of overlapping signaling networks. Both cAMP-dependent signaling and Snail transcription factor appears to be central to both neuroendocrine differentiation and EMT in prostate cancer.

At the ASCO 2015 meeting, Small, *et al.* reported detailed pathological and molecular characterization of prostate cancer metastases resistant to enzalutimide or abiraterone, two new drugs which are very effective at blocking the androgen receptor or blocking testosterone synthesis, respectively [5, 6]. Pure neuroendocrine differentiation was seen in 13%, while 26% were a mixture of neuroendocrine and adenocarcinoma histology. An additional 26% exhibited a new pathology, which they named "intermediate atypical carcinoma" as at a molecular level it represented a transition between the adenocarcinoma and neuroendocrine states. Only 35% exhibited the classic adenocarcinoma on a histologic and a molecular basis. At the same meeting, Beltran, *et al.* showed that the prostate neuroendocrine biopsies showed changes in genes involved in cell-cell adhesion, EMT, neuronal differentiation, homeobox, synapse or organ morphogenesis [7].

These reports show that molecular events associated neuroendocrine differentiation and EMT can be coexpressed at the clinical level. Additionally, neuroendocrine differentiation appears to be relatively common in patients who progress on the newer highly potent androgen receptor antagonists or androgen synthesis inhibitors. Small, *et al.* observed neuroendocrine differentiation at some level in 65% of all such patients. This finding underlines the importance of developing successful strategies to either prevent or treat neuroendocrine differentiation as well as EMT. As we will discuss, it may be possible to block both neuroendocrine differentiation and epithelial to mesenchymal transition in prostate cancer.

NEUROENDOCRINE DIFFERENTIATION

Neuroendocrine differentiation is not a major feature of prostate cancer at diagnosis. It is most commonly encountered after a period of androgen withdrawal associated with the appearance of hormone-resistance. This has naturally led to a focus on the interaction between androgen signaling and the neuroendocrine phenotype. A number of interesting interactions have been documented.

When the androgen-responsive human prostate cancer cell line, LNCAP, is cultured in under low androgen conditions, the cells undergo neuroendocrine differentiation. This reverses after the addition of dihydrotestosterone. Inhibition of AR expression with siRNA also induces neuroendocrine differentiation [8, 9].

The literature linking cAMP-dependent signaling with neuroendocrine differentiation in prostate cancer

dates to 1994 when our group showed that stable cAMP analogs and phosphodiesterase inhibition triggered neuroendocrine differentiation in prostate cancer cells [10]. The next critical observation was the report by Cox, *et al.* that transfection with a constitutively active catalytic PKA was sufficient to create prostate cancer cell lines that showed stable neuroendocrine differentiation [11, 12]. Conversely, transfection with the regulatory subunit of PKA was sufficient to block neuroendocrine differentiation in the same prostate cancer cell lines. Furthermore, adding the cells transfected with constitutively active PKA were mixed with cells showing the adenocarcinoma phenotype, proliferation of the later were stimulated. Thus, while neuroendocrine differentiation was associated with a drop in proliferation, in a mixed population of cells, cancer growth was enhanced [13].

Neuroendocrine differentiation can also be induced by molecules like VIP, PTHrP and catacholamines known to act *via* heterotrimeric G-protein coupled receptors that exert their biologic impact at least in part *via* PKA [14]. Furthermore, alterations in expression of PKA subunit catalytic and regulatory isoforms, adenylyl cyclase isoforms and expression of specific phosphodiesterase isoforms appear during prostate cancer progression and have prognostic significance.

The signaling pathway by which PKA trigger neuroendocrine differentiation has been partially elucidated. There is convincing evidence that suppression of RhoA activity plays an essential role [15]. Transfection with the constitutively active Gln63Leu-mutated RhoA acted as a dominant-negative inhibitor for cAMP-driven neuroendocrine differen-tiation. An increase in cAMP results in PKA-dependent phosphorylation of RhoA at serine 188, decreasing its activity. A mutant RhoA lacking serine 188 blocked cAMP-dependent neuroendocrine differentiation. These results suggest that inactivation of RhoA by PKA is a key step in neuroendocrine differentiation induced by PKA.

Overexpression of the snail transcription factor suppresses E-cadherin expression and induces EMT in a range of cell models. Prostate cancer responds similarly and undergoes EMT associated with snail overexpression. However, snail overexpression in prostate cancer also induces neuroendocrine differentiation, including release of paracrine growth factors and release of chromogranin A [16, 17]. Furthermore, suppression of snail by siRNA reverses neuroendocrine differentiation induced by either snail overexpression or androgen withdrawal.

Is there any evidence that Snail-induced changes in prostate cancer are linked with cAMP and PKA activity?

TGF beta can promote EMT and PKA appears essential in this process *via* its interaction with Smad-4. In 1992, our group reported than treatment of prostate cancer cells with cAMP analog dibutyryl cAMP let to increased production of TGF beta 2 [18]. TGF-beta-2 is a well-established trigger for EMT induction and has been reported to increase Snail expression [19].

Snail activity is increased by phosphorylation of serine 11 and 92 and this phosphorylation is accomplished by PKA for serine 11 and casein kinase-2 at serine 92 [20]. This phosphorylation both increases snail stability and enhances snail function.

Thus, in prostate cancer cells, cAMP signaling foster neuroendocrine differentiation and EMT in a Snail-dependent manner.

cAMP/PKA AS A THERAPEUTIC TARGET

The evidence supports a role for cAMP in both neuroendocrine differentiation and in EMT through TGF beta-2 and Snail. However, cAMP is involved in a wide range of physiologic processes that depend on heterotrimeric G protein signaling events. Global blockade of cAMP-dependent signaling events would pose a high risk of toxicity.

Problems similar to this are common in drug discovery. The development of adrenergic receptor agonists and antagonists was initially problematic because of the diverse roles of norepinephrine and epinephrine play in normal physiology. This issue was solved by the discovery of multiple alpha- and beta-adrenergic receptors. It allowed the development of agonists and antagonists specific to the various receptors and a marked improvement in therapeutic efficacy. There may be similar opportunities for tissue- or cancer-specific inhibition of cAMP dependent induction of neuroendocrine differentiation and EMT.

PKA ISOTYPE IN PROSTATE CANCER

PKA is one of the best-characterized protein kinases. The details of this enzyme and its regulation have been ably reviewed elsewhere [21]. In the resting state, it exists with the catalytic subunits bound to a regulatory unit. The complex of regulatory and catalytic subunits is typically found bound to an anchoring protein, AKAP, which restricts the subcellular

distribution of PKA activity and appears to be important in limiting the range of possible biochemical consequences of PKA activation. Upon activation by cAMP, the catalytic subunit dissociates from the regulatory subunit bound to AKAP.

There are specific alterations in the PKA and AKAP isoforms involved in prostate cancer [22-25]. The regulator subunit has two isoforms, RI and RII. In a clinical trial, expression of RI alpha was adversely associated with the outcome of radiation therapy for prostate cancer. In contrast, RII alpha expression was associated with increased responsiveness to taxane chemotherapy. Antisense inhibition of R1 alpha resulted in growth arrest in colon and prostate cancer. This inhibition of the regulatory subunit was associated with nuclear translocation of the catalytic subunit. These studies did not examine associated changes in either neuroendocrine differentiation or EMT. However, Cox *et al.* showed that transfection with active catalytic subunit CIalpha is sufficient to cause stable neuroendocrine differentiation and this was blocked by transfection with the dominant negative R1 alpha subunit [12].

The catalytic beta subunit of PKA exists in several splice variants. During prostate cancer neuroendocrine conversion, C beta 2 is reduced in expression, while C beta 1, 3 and 4 are increased [26]. Thus a drop in C beta 2 may be characteristic of the adenocarcinoma phenotype, while C beta 1, 3 and 4 with the neuroendocrine phenotype.

As mentioned earlier, AKAP dictates the subcellular localization of PKA. AKAP also exists in several isoforms and increased expression of AKAP-4 is common in prostate cancer and other malignancies [27]. We couldn't find any reports on the impact of AKAP-4 on subcellular localization of PKA, but examining the impact of AKAP-4 would appear to be essential in understanding the impact of cAMP signaling events in prostate cancer.

The structure of PKA is well established and inhibitors have been developed. Balanol has attracted considerable attention [28-31]. This fungal product inhibits both PKA and PKC with a Ki in the nanomolar range. It acts as a competitive antagonist of ATP binding to the calalytic subunit. Analogs have been produced with high specificity for either PKA or PKC. As far as we can determine, no PKA has undergone clinical trial testing. Because PKA is involved in so many normal physiologic processes, inhibition of PKA would be associated with high risk of unacceptable side effects unless the inhibition were limited in a tissue and/or cancer specific manner.

ADENYLYL CYCLASE ISOFORMS IN PROSTATE CANCER

Adenylyl cyclase catalyzes the conversion of ATP to cAMP and is critical to the activation of PKA. There are 10 known isoforms of adenylyl cyclase in mammalian tissue [32-34]. Nine possess transmembrane domains and are involved in heterotrimeric G-protein-coupled signaling. The tenth isoform, called soluble adenylyl cyclase (sAC) lacks transmembrane domains and is found in the cytosol, nucleus, mitochondria and centriole [35, 36]. Soluble adenylyl cyclase is not involved in heterotrimergic G-protein coupled signaling. Instead, it is activated by bicarbonate and calcium. Soluble adenylyl cyclase is found in a range of normal tissues, including sperm, neutrophils, brain, kidney, eye and pancreas. It has been found to undergo nuclear translocation in both skin squamous carcinoma and melanoma.

Normal and malignant prostate cells poses both membrane-bound and soluble adenylyl cyclase. The former is involved in G-protein coupled response of these cells to a range of compounds, including PTHrP, VIP and epinephrine. Until recently, the role of soluble adenylyl cyclase in prostate cancer was poorly understood. Overexpression of soluble adenylyl cyclase is found in prostate cancer cells as compared with normal prostate cells [37, 38]. Through the use of small molecule inhibitors and knockdown with siRNA, the function of soluble adenylyl cyclase has been clarified. Inhibition of soluble adenylyl cyclase results in a marked decline in proliferation and the onset of mitochondrial apoptosis.

In addition to PKA, EPAC (exchange protein activated by cAMP) is another main downstream target of cAMP. In prostate cancer cells, soluble adenylyl cyclase appears to signal through EPAC/RAP1/B-RAF rather than PKA as stimulation [36]. Additionally, inhibition of PKA failed to alter events triggered by activation of soluble adenylyl cyclase.

These results point to a complex role of cAMP in prostate cancer biology. Results discussed earlier in this review point to a role of G-protein-coupled activation of transmembrane adenylyl cyclase in the activation of PKA contributing to neuroendocrine differentiation and modulation of the epithelial/

mesenchyme transition. In contrast, soluble adenylyl cyclase appears to be involved in the regulation of proliferation and mitochondrial apoptosis.

These two signaling pathways differ markedly in their interaction with androgen-dependent signaling in prostate cancer. The PKA-dependent pathway is activated by manipulations that suppress androgen signaling. This PKA pathway has, in turn, been shown to be sufficient to trigger neuroendocrine conversion and facilitate EMT. In contrast, soluble adenylyl cyclase signaling appears to be independent of androgen and has not been implicated in either neuroendocrine differentiation or EMT.

The functional and structural differences between the membrane bound and soluble adenylyl cyclase are sufficient that it has been possible to develop inhibitors with relative specificity for these two distinct isoforms. Already, progress has been made in developing inhibitors specific to soluble as compared to membrane-bound adenylyl cyclase [33, 39]. Additionally, there has been progress in developing inhibitors for the various isoforms of the membrane-bound adenylyl cyclases. This opens the way to probe further the roles of these two distinct cAMP-dependent signal transduction pathways in prostate cancer biology. With this in mind, we were unable to find any documentation of the specific transmembrane adenylyl cyclases expressed in prostate cancer.

The diversity of adenylyl cyclases, the restricted tissue distribution of the various isoforms and the existence of isoform-specific inhibitors would appear to make adenylyl cyclase a sufficiently promising therapeutic target to warrant further investigation.

CYCLIC NUCLEOTIDE PHOSPHODIESTERASE ISOFORMS IN PROSTATE CANCER

Cyclic nucleotide phosphodiesterase is currently the only known pathway for cAMP degradation. This enzyme family is characterized by multiple isoforms that allow for tissue-specific regulation of cAMP degradation. Furthermore, these isoforms additionally foster intracellular compartmentalization both spatially and temporally allow multiple cAMP-dependent signaling events to occur with a degree of independence.

The pattern of isoform expression in prostate cancer has been studied. The expression of one isoform, PDE4D7, appears to be tightly linked to the appearance of hormone-resistant prostate cancer [40, 41]. PDE4D7 exists in both membrane-bound and free in the cytosol. There is a marked reduction in PDE4D7 expression at the RNA and protein level in androgen resistant as compared to androgen sensitive prostate cancer cells. Furthermore, in hormone sensitive prostate cancer, PDE4D7 accounts for a majority of membrane bound cAMP hydrolytic capacity in hormone sensitive prostate cancer cells. When membrane association of PDE4D7 is blocked, there is an increase in proliferation rate in hormone sensitive prostate cancer. All of this evidence supports a role for membrane-bound PDE47D cAMP hydrolysis as a factor limiting proliferation in hormone sensitive cells and its decrease as a factor supporting proliferation in the androgen-independent state.

These results would appear inconsistent with other observations discussed above on soluble adenylyl cyclase were cytosolic cAMP fosters proliferation and resistance to mitochondrial apoptosis. Furthermore, membrane-bound adenylyl cyclase was found to activate PKA and neuroendocrine differentiation: neuroendocrine differentiation is associated with prolix-feration arrest. However, cAMP signaling is complex with multiple levels of regulation and subcellular compartmentalization. As cAMP downstream events in prostate cancer may well reflect the balance between PKA and EPAC signaling pathways, it might prove fruitful to monitor the activity of these two pathways as activity and membrane localization of PDE4D7 is modulated.

As a reduction in PDE4D7 activity enhances proliferation, it is tempting to consider strategies that might increase PDE4D7 activity. However, increased activity of the PDE4 family is characteristic of many disease processes and the development of antagonists rather than agonists of this phosphodiesterase family dominate drug development. Unless the increase in PDE4 activity can be limited to prostate tissue, it has a high risk of exacerbating a range of other disease processes.

CONCLUSIONS

Activation of PKA by cAMP is sufficient to trigger neuroendocrine differentiation and EMT in prostate cancer cells. Also, in prostate cancer, both neuroendo-crine differentiation and EMT appear to be mediated by Snail and cAMP has been implicated in Snail function. Thus, inhibitors cAMP-dependent signaling represent a possible approach to simultaneously block both neuroendocrine differentiation and EMT in prostate

cancer. However, cAMP is central to the function of a wide range of heterotrimeric G-protein linked receptors essential to normal tissue function. Thus, global blockade of cAMP signaling would likely be associated with serious toxicity.

The key proteins involved in cAMP signaling exist in multiple isoforms that serve to limit activity of this pathway in time and space. It is possible that inhibitors selective for isoforms specific to prostate might sufficiently limit the impact on normal tissues allow the development of clinically useful agents. While key details are lacking, adenylyl cyclase appears to be an attractive target.

REFERENCES

[1] Crawford ED, Eisenberger MA, McLeod DG, *et al*. A controlled trial of leuprolide with and without flutamide in prostatic carcinoma. N Engl J Med 1989; 321: 419-424. http://dx.doi.org/10.1056/NEJM198908173210702

[2] Cerasuolo M, Paris D, Iannotti FA, *et al*. Neuroendocrine Transdifferentiation in Human Prostate Cancer Cells: An Integrated Approach. Cancer Res 2015; 75: 2975-2986. http://dx.doi.org/10.1158/0008-5472.CAN-14-3830

[3] Berman-Booty LD, Knudsen KE. Models of neuroendocrine prostate cancer. Endocr Relat Cancer 2015; 22: R33-49. http://dx.doi.org/10.1530/ERC-14-0393

[4] Parimi V, Goyal R, Poropatich K, Yang XJ. Neuroendocrine differentiation of prostate cancer: a review. Am J Clin Exp Urol 2014; 2: 273-285.

[5] Eric Jay Small JH, Jack Youngren *et al*. Characterization of neuroendocrine prostate cancer (NEPC) in patients with metastatic castration resistant prostate cancer (mCRPC) resistant to abiraterone (Abi) or enzalutamide (Enz): Preliminary results from the SU2C/PCF/AACR West Coast Prostate Cancer Dream Team (WCDT). J Clin Oncol 2014; 32: (suppl 4; abstr 79).

[6] Small EJ, Huang J, Youngren J, *et al*. Characterization of neuroendocrine prostate cancer (NEPC) in patients with metastatic castration resistant prostate cancer (mCRPC) resistant to abiraterone (Abi) or enzalutamide (Enz): Preliminary results from the SU2C/PCF/AACR West Coast Prostate Cancer Dream Team (WCDT). J Clin Oncol 2015; 33: (suppl; abstr 5003).

[7] Beltran H, Prandi H, Mosquera JM, *et al*. Defining a molecular subclass of treatment resistant prostate cancer. J Clin Oncol 2015; 33: (suppl; abstr 5004)

[8] Yuan TC, Veeramani S, Lin FF, *et al*. Androgen deprivation induces human prostate epithelial neuroendocrine differentiation of androgen-sensitive LNCaP cells. Endocr Relat Cancer 2006; 13: 151-167. http://dx.doi.org/10.1677/erc.1.01043

[9] Jongsma J, Oomen MH, Noordzij MA, *et al*. Androgen-independent growth is induced by neuropeptides in human prostate cancer cell lines. Prostate 2000; 42: 34-44. http://dx.doi.org/10.1002/(SICI)1097-0045(20000101)42:1<34::AID-PROS5>3.0.CO;2-2

[10] Bang YJ, Pirnia F, Fang WG, *et al*. Terminal neuroendocrine differentiation of human prostate carcinoma cells in response to increased intracellular cyclic AMP. Proc Natl Acad Sci USA 1994; 91: 5330-5334. http://dx.doi.org/10.1073/pnas.91.12.5330

[11] Cox ME, Deeble PD, Lakhani S, Parsons SJ. Acquisition of neuroendocrine characteristics by prostate tumor cells is reversible: implications for prostate cancer progression. Cancer Res 1999; 59: 3821-3830.

[12] Cox ME, Deeble PD, Bissonette EA, Parsons SJ. Activated 3',5'-cyclic AMP-dependent protein kinase is sufficient to induce neuroendocrine-like differentiation of the LNCaP prostate tumor cell line. J Biol Chem 2000; 275: 13812-13818. http://dx.doi.org/10.1074/jbc.275.18.13812

[13] Deeble PD, Cox ME, Frierson HF, *et al*. Androgen-independent growth and tumorigenesis of prostate cancer cells are enhanced by the presence of PKA-differentiated neuroendocrine cells. Cancer Res 2007; 67: 3663-3672. http://dx.doi.org/10.1158/0008-5472.CAN-06-2616

[14] Alberti C. Neuroendocrine differentiation in prostate carcinoma: focusing on its pathophysiologic mechanisms and pathological features. G Chir 2010; 31: 568-574.

[15] Jones SE, Palmer TM. Protein kinase A-mediated phosphorylation of RhoA on serine 188 triggers the rapid induction of a neuroendocrine-like phenotype in prostate cancer epithelial cells. Cell Signal 2012; 24: 1504-1514. http://dx.doi.org/10.1016/j.cellsig.2012.03.018

[16] McKeithen D, Graham T, Chung LW, Odero-Marah V. Snail transcription factor regulates neuroendocrine differentiation in LNCaP prostate cancer cells. Prostate 2010; 70: 982-992. http://dx.doi.org/10.1002/pros.21132

[17] Smith BN, Odero-Marah VA. The role of Snail in prostate cancer. Cell Adh Migr 2012; 6: 433-441. http://dx.doi.org/10.4161/cam.21687

[18] Bang YJ, Kim SJ, Danielpour D, *et al*. Cyclic AMP induces transforming growth factor beta 2 gene expression and growth arrest in the human androgen-independent prostate carcinoma cell line PC-3. Proc Natl Acad Sci USA 1992; 89: 3556-3560. http://dx.doi.org/10.1073/pnas.89.8.3556

[19] Kokudo T, Suzuki Y, Yoshimatsu Y, Yamazaki T, Watabe T, Miyazono K. Snail is required for TGFbeta-induced endothelial-mesenchymal transition of embryonic stem cell-derived endothelial cells. J Cell Sci 2008; 121: 3317-3324. http://dx.doi.org/10.1242/jcs.028282

[20] MacPherson MR, Molina P, Souchelnytskyi S, *et al*. Phosphorylation of serine 11 and serine 92 as new positive regulators of human Snail1 function: potential involvement of casein kinase-2 and the cAMP-activated kinase protein kinase A. Mol Biol Cell 2010; 21: 244-253. http://dx.doi.org/10.1091/mbc.E09-06-0504

[21] Rinaldi L, Sepe M, Donne RD, Feliciello A. A dynamic interface between ubiquitylation and cAMP signaling. Front Pharmacol 2015; 6: 177. http://dx.doi.org/10.3389/fphar.2015.00177

[22] Cho-Chung YS, Nesterova MV. Tumor reversion: protein kinase A isozyme switching. Ann NY Acad Sci 2005; 1058: 76-86. http://dx.doi.org/10.1196/annals.1359.014

[23] Kvissel AK, Ramberg H, Eide T, Svindland A, Skålhegg BS, Taskén KA. Androgen dependent regulation of protein kinase A subunits in prostate cancer cells. Cell Signal 2007; 19: 401-409. http://dx.doi.org/10.1016/j.cellsig.2006.07.011

[24] Neary CL, Cho-Chung YS. Nuclear translocation of the catalytic subunit of protein kinase A induced by an antisense oligonucleotide directed against the RIalpha regulatory subunit. Oncogene 2001; 20: 8019-8024. http://dx.doi.org/10.1038/sj.onc.1204992

[25] Nesterova M, Noguchi K, Park YG, Lee YN, Cho-Chung YS. Compensatory stabilization of RIIbeta protein, cell cycle deregulation, and growth arrest in colon and prostate carcinoma cells by antisense-directed down-regulation of protein kinase A RIalpha protein. Clin Cancer Res 2000; 6: 3434-3441.

[26] Cho YS, Cho-Chung YS. Antisense protein kinase A RIalpha acts synergistically with hydroxycamptothecin to inhibit growth and induce apoptosis in human cancer cells: molecular basis for combinatorial therapy. Clin Cancer Res 2003; 9: 1171-1178.

[27] Chiriva-Internati M, Yu Y, Mirandola L, *et al*. Identification of AKAP-4 as a new cancer/testis antigen for detection and immunotherapy of prostate cancer. Prostate 2012; 72: 12-23. http://dx.doi.org/10.1002/pros.21400

[28] Akamine P, Madhusudan, Brunton LL, *et al*. Balanol analogues probe specificity determinants and the conformational malleability of the cyclic 3',5'-adenosine monophosphate-dependent protein kinase catalytic subunit. Biochemistry 2004; 43: 85-96. http://dx.doi.org/10.1021/bi035042p

[29] Gustafsson AB, Brunton LL. Differential and selective inhibition of protein kinase A and protein kinase C in intact cells by balanol congeners. Mol Pharmacol 1999; 56: 377-382.

[30] Pande V, Ramos MJ, Gago F. The Protein kinase inhibitor balanol: structure-activity relationships and structure-based computational studies. Anticancer Agents Med Chem 2008; 8: 638-645. http://dx.doi.org/10.2174/187152008785133056

[31] Wong CF, Hünenberger PH, Akamine P, *et al*. Computational analysis of PKA-balanol interactions. J Med Chem 2001; 44: 1530-1539. http://dx.doi.org/10.1021/jm000443d

[32] Kamenetsky M, Middelhaufe S, Bank EM, Levin LR, Buck J, Steegborn C. Molecular details of cAMP generation in mammalian cells: a tale of two systems. J Mol Biol 2006; 362: 623-639. http://dx.doi.org/10.1016/j.jmb.2006.07.045

[33] Iwatsubo K, Tsunematsu T, Ishikawa Y. Isoform-specific regulation of adenylyl cyclase: a potential target in future pharmacotherapy. Expert Opin Ther Targets 2003; 7: 441-451. http://dx.doi.org/10.1517/14728222.7.3.441

[34] Patel TB, Du Z, Pierre S, Cartin L, Scholich K. Molecular biological approaches to unravel adenylyl cyclase signaling and function. Gene 2001; 269: 13-25. http://dx.doi.org/10.1016/S0378-1119(01)00448-6

[35] Kleinboelting S, Diaz A, Moniot S, *et al*. Crystal structures of human soluble adenylyl cyclase reveal mechanisms of catalysis and of its activation through bicarbonate. Proc Natl Acad Sci USA 2014; 111: 3727-3732. http://dx.doi.org/10.1073/pnas.1322778111

[36] Steegborn C. Structure, mechanism, and regulation of soluble adenylyl cyclases - similarities and differences to transmembrane adenylyl cyclases. Biochim Biophys Acta 2014; 1842: 2535-2547. http://dx.doi.org/10.1016/j.bbadis.2014.08.012

[37] Flacke JP, Flacke H, Appukuttan A, *et al*. Type 10 soluble adenylyl cyclase is overexpressed in prostate carcinoma and controls proliferation of prostate cancer cells. J Biol Chem 2013; 288: 3126-3135. http://dx.doi.org/10.1074/jbc.M112.403279

[38] Appukuttan A, Flacke JP, Flacke H, Posadowsky A, Reusch HP, Ladilov Y. Inhibition of soluble adenylyl cyclase increases the radiosensitivity of prostate cancer cells. Biochim Biophys Acta 2014; 1842: 2656-2663. http://dx.doi.org/10.1016/j.bbadis.2014.09.008

[39] Brand CS, Hocker HJ, Gorfe AA, Cavasotto CN, Dessauer CW. Isoform selectivity of adenylyl cyclase inhibitors: characterization of known and novel compounds. J Pharmacol Exp Ther 2013; 347: 265-275. http://dx.doi.org/10.1124/jpet.113.208157

[40] Byrne AM, Elliott C, Hoffmann R, Baillie GS. The activity of cAMP-phosphodiesterase 4D7 (PDE4D7) is regulated by protein kinase A-dependent phosphorylation within its unique N-terminus. FEBS Lett 2015; 589: 750-755. http://dx.doi.org/10.1016/j.febslet.2015.02.004

[41] Henderson DJ, Byrne A, Dulla K, *et al*. The cAMP phosphodiesterase-4D7 (PDE4D7) is downregulated in androgen-independent prostate cancer cells and mediates proliferation by compartmentalising cAMP at the plasma membrane of VCaP prostate cancer cells. Br J Cancer 2014; 110: 1278-1287. http://dx.doi.org/10.1038/bjc.2014.22

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