A review of orexin’s unprecedented potential as a novel, highly-specific treatment for various localized and metastatic cancers

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Abstract
A systematic review was conducted to categorize the types of cancerous tissues that express orexin receptors and also to examine the effect of in vitro administration of orexin A or B to corresponding cell samples. Comprehensive literature analyses of primary experimental studies were performed. The results of the review included an increased frequency of orexin receptor expression in many colon and prostate cancer tissues and an upward trend of pro-apoptotic activity in these aggressive cell types.

Keywords
Cancer, orexin, hypocretin, apoptosis, orexin receptor, orexin 1 receptor, orexin 2 receptor, orexin A, orexin B

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Background
Recently published in the *Journal of Biomedical Science* (March, 2017) was the review article entitled “Evaluation of the use of therapeutic peptides for cancer treatment.” Notably absent from this review was data from two peptides which have been regularly researched for anti-cancer effects, orexin A (OXA), and orexin B (OXB). These neurotransmitters, also known as hypocretins, have incited enthusiasm in the field of cancer research as promoters of apoptosis in various colon, prostate, and hepatic cancer cell lines. Therefore, the prospect of identifying the role of orexin peptides as a potential new treatment modality for cancer patients is appealing. Furthermore, studies that reveal orexins’ effect in cancer cell death in later-stage metastatic colon cancer encourages research models that target this patient population, which typically faces limited treatment options. It is important to note, however, that these endogenous peptides have a relatively short plasma half-life of approximately 27 min, along with conflicting data of the effects of orexins in certain malignant cell types. Therefore, a closer examination of the potential limitations of the use of this treatment within certain conditions is required. This review of current research focuses on the expression of orexin receptors and the consequences of receptor stimulation in transformed cells with the objective of providing in guidance for future orexin research and to promote a currently hypothetical, but highly specific cure for certain localized and metastatic solid tumors originating in the colon and prostate.

Orexins are produced in the hypothalamic region of the brain and act upon two specific G protein–coupled receptors found on the surface of native neurons in the central nervous system (CNS), namely, orexin 1 receptor (OX1R also known as HCRT1) and orexin 2 receptor (OX2R also known as HCRT2). Occupation of these receptors regulates several important physiological functions, including the sleep–wake cycle, energy metabolism, and feeding behaviors as listed in Table 1. While OXA binds to both receptors with a high affinity, OXB only binds to OX2R with a high affinity and to OX1R with a markedly reduced affinity.

OXA is a large, lipophilic polypeptide (MW=3562) which effluxes from the brain to the systemic blood circulation at a rate similar to that of albumin. It has been shown to have an influx...
Table 1. Summary of orexin receptor effects in the central nervous system.

| Receptor | Proposed effect of stimulation                                      | Proposed effect of antagonism                        | Binding affinity |
|----------|---------------------------------------------------------------------|------------------------------------------------------|------------------|
| OX1R     | Reward-seeking                                                     | Anti-addiction                                       | +++              |
|          | Anxiety                                                             | Anxiolytic                                           | +/–              |
|          | ↑ levels of amyloid beta (theoretically increasing risk of Alzheimer’s disease) | ↓ levels of amyloid beta (theoretically anti-Alzheimer’s disease) | +/–              |
|          | Wakefulness                                                         | Narcolepsy                                           | ++               |
| OX2R     | Insomnia                                                            | Sleep/cataplex                                       | +++              |
|          | Increased food intake (short-term)                                  | Anorexia (short-term)                                | +++              |
|          | Anti-obesity and anti-insulin-resistance effects by improving leptin sensitivity (long-term) | High-fat diet–induced obesity and insulin resistance (long-term) | +++              |

OX1R: orexin 1 receptor; OX2R: orexin 2 receptor.

*Adapted from the study of Kodadek et al. and references therein.

The profound effect of orexin interaction with malignant cells was first discovered as a result of an in vitro study in 2004 that demonstrated apoptosis in colon cancer cells subsequent to orexin treatment. It was discovered here that certain colon cancers ectopically express orexin receptors and stimulation of these receptors triggers robust apoptosis of the host cell. Many further studies tested the frequency of orexin receptor expression in a wide variety of cancer cells and sought to determine the effect of orexin stimulation of these receptors. One experimental model included the subcutaneous inoculation of LoVo colon cancer cells in nude mice, which lead to tumor formations. The mice were then treated daily for a period of 15 days with 1.12 μmol/kg intraperitoneal injections of OXA. The subsequent results were astonishing with an 80% average reduction in tumor size and were reproduced with similar results in HT29-derived colon tumors. No apparent side effects were noted in the study animals, which corroborates the theory that a lack of orexin receptor expression in the periphery may naturally limit the possible adverse effects that would result from future in vivo orexin treatment models.

As orexin research has progressed over the years, it was discovered that not only are orexin receptors ectopically expressed in colon cancer cells but it appears that orexin receptor expression is upregulated in later-staged metastatic cells. Further, in hepatic metastases of colon cancer, the presence of OX1Rs was confirmed only on the surface of cancerous cells and not on surrounding hepatocytes, which is consistent with previous observations in localized colon cancer tissues versus surrounding non-transformed tissues. This finding is significant because current treatments for metastatic cancers are often limited by systemic toxicity, which may be potentially ameliorated with orexin treatment due to a lack of receptors in the normal tissues surrounding tumors. Given this, orexin therapy in cancer patients has the potential of becoming an unprecedented, highly-specific treatment option with limited adverse effects.

Similar to its effects in colon cancer cells, orexin also promotes apoptosis in prostate cancer cells. Because there are many studies involving orexin receptors in various types of cancers, it is important to analyze the presence and extent of orexin receptor expression in all cancers cell types. The only prior literature review of orexin receptors and cancer tissue types known to us is a 2011 review focused on an overview of orexin’s role in colon cancer cells. Therefore, the purpose of the present review is to provide a comprehensive analysis of published studies which details all cancerous tissues tested for orexin receptor expression and the effects of orexin stimulation of these receptors, if tested.

Methods

The systematic scientific literature search for this reporting included the PubMed database with the search algorithm: (orexin or hypocretin) AND cancer (last search date: 28 March 2017). A second search was performed using the EBSCO database using the same search algorithm: (orexin...
or hypocretin) AND cancer (last search date: 28 March 2017). All available literatures were searched, which yielded 98 unique references. These references were screened for full-text review based on a priori inclusion criteria. Inclusion parameters required primary experimental studies of in vivo cancer models or in vitro cancerous tissues that identified cells expressing native (non-transfected) orexin receptors and/or studies that tested the effect of the application of orexin to cancer tissues. Cancerous tissues included experimental cell lines, cell models, or clinical samples. References from identified studies were screened by title or abstract for inclusion based on the a priori inclusion parameters applied to the original search articles (see Figure 1). All studies were assessed for potential bias based on funding or author affiliations.

**Results**

**Orexin receptor expression in colon cancers**

OX1R messenger RNA (mRNA) was expressed in all clinical colon cancer samples tested and in the following cell lines: HT-29, HT29-FU, HT29-D4, SW48, SW620, SW480, Caco-2, LoVo, Colo205, T84, and LS174T. OX1 mRNA was not expressed in HCT-116 cell lines. OX2R mRNA was not expressed in any of the cell lines tested.

OXA and OXB induced significant apoptosis in the following cell lines: SW48, SW620, SW480, Caco-2, LoVo, Colo205, T84, and LS174T. OXB reduced cell production in the following cell lines: SW480, Caco-2, and LoVo. OXA drastically reduced tumor size in LoVo and HT29 xenograft tumors, while no effect was observed in HCT-116 xenograft tumors. OXA had a variable response in the HCT-116 cell line, which included induced autophagy (resistance to apoptosis), reduced cell viability, apoptosis promotion, and no significant effect. OXB had no significant effect on the HCT-116 cell line. OXA and OXB induced significant apoptosis and inhibited cell growth in HT-29 and HT29-DR cell lines. OXA and OXB induced significant apoptosis in the HT29-FU cell line.

**Orexin receptor expression and effects on gastric cancers**

OX1R mRNA was expressed in the following cell lines: BGC-823 and SGC-7901. OX2R mRNA was not expressed in either of these cell lines.
OXA increased OX1R expression and increased cell proliferation and viability by 150% in the BGC-823 cell line. OXA stimulated cell proliferation and viability in the SGC-7901 cell line.

**Orexin receptor expression in hepatic cancers**

OX1R mRNA was expressed in 28 of 41 clinical hepatocellular cancer samples as well as in the Hep3B cell line. Neither the sample tissues nor the cell line tested positive for OX2R mRNA expression.

OXA increased glucose uptake in the Hep3B cell line.

**Orexin receptor expression in prostate cancers**

OX1R mRNA was expressed in all clinical prostate cancer samples tested. The expression of OX1R mRNA was variable in LNCaP, DU145, and DU145 cell lines. OX1R mRNA was not found in the following cell lines: PC3, PrEC, PrSmC, PrSc, or any clinical normal and benign prostatic hyperplasia (BPH) sample tissues tested. OX2R mRNA was expressed in all clinical and BPH sample tissues, but was not expressed in LNCaP, DU145, PC3, PrEC, PrSmC, or PrSc cell lines.

OXA significantly decreased cell survival and significantly reduced androgen receptor nuclear translocation in the presence of testosterone in the LNCaP cell line.

**Table 2. Orexin receptor mRNA expression in cancer cells.**

| System                      | Organ         | Cell type                          | OX1R | OX2R |
|-----------------------------|---------------|------------------------------------|------|------|
| Digestive system            | Colon         | 38 of 38 clinical colon cancer samples +   |      |      |
|                             |               | 10 of 10 clinical liver metastases of colon cancer samples +   |      |      |
|                             |               | HT-29   +                        |      |      |
|                             |               | HT29-FU +                        |      |      |
|                             |               | HT29-D48 +                       |      |      |
|                             |               | SW482 +                          |      |      |
|                             |               | SW6202 +                         |      |      |
|                             |               | SW4802 +                         |      |      |
|                             |               | Caco-2 +                          |      |      |
|                             |               | LoVo +                            |      |      |
|                             |               | Colo2052 +                       |      |      |
|                             | HT29 +         |                              |      |      |
| Stomach                     | BGC-823       | +                                 |      |      |
|                             | SGC-7901      | +                                 |      |      |
| Liver                       | Hep3B14       | +                                 |      |      |
|                             | 28 of 41 clinical hepatocellular cancer samples + |      |      |
| Endocrine/reproductive      | Prostate      | LNCaP9,15,16 +/–                  |      |      |
| system                      |               | DU1459,16 +/–                     |      |      |
|                             |               | PC316 +                           |      |      |
|                             |               | PrEC16 –                          |      |      |
|                             |               | PrSmC16 –                         |      |      |
|                             |               | PrSc16 –                          |      |      |
|                             |               | 15 of 15 clinical prostate cancer samples9 + |      |      |
|                             |               | 30 of 30 clinical normal and BPH samples16 – |      |      |
|                             | Cervix         | 45 of 47 clinical cervical cancer samples17 + |      |      |
|                             | Adrenal gland (cortex) | 10 of 10 clinical samples of cortisol-secreting adenomas7 + |      |      |
|                             | Adrenal gland (medullary) | NCI-H295R21,22 +/–          |      |      |
|                             | Central nervous system | Brain | Rat glioma C623 + |      |      |
|                             | Peripheral nervous system | Nerve tissues | SK-N-MC24 – |      |      |
| Stem cells                  | CD34+ Clinical samples of CML25 + |      |      |

OX1R: orexin 1 receptor; OX2R: orexin 2 receptor; PC: pheochromocytoma; CML: chronic myelogenous leukemia; BPH: benign prostatic hyperplasia.

+Originally compiled summary of data from references cited within table.
and OXB increased cell growth in non-differentiated cells and induced significant apoptosis in differentiated cells in DU145 cell lines.

**Orexin receptor expression in cervical cancers**

OX1R and OX2R mRNA was expressed in 45 of 47 clinical cervical cancer samples.

**Orexin receptor expression in endometrial cancers**

OXA and OXB resulted in no observed apoptosis effect in the following cell lines: ECC-1, Ishikawa, and MFE-280.

**Orexin receptor expression in adrenal cancers**

Cancers of the adrenal cortex. OX1R and OX2R mRNA was not expressed in rat pheochromocytoma (PC), PC12. OX2R mRNA was expressed in all human PC clinical samples tested, but OX1R mRNA was not.

OXA and OXB increased basal cortisol levels and proliferation in clinical samples of adenomas. OXA increased cortisol secretion, down-regulated OX2R mRNA, increased OX1R mRNA, and enhanced cell proliferation in the NCI-H295R cell line.

Cancers of the adrenal medulla. OX1R and OX2R mRNA was expressed on all clinical cortisol-secreting adenoma samples tested. OX1R and OX2R mRNA expression was variable for the NCI-H295R cell line.

OXA and OXB decreased tyrosine hydroxylase production in the PC12 cell line and increased inositol triphosphate (IP3), epinephrine, and norepinephrine release in clinical samples of PC.

**Orexin receptor expression in brain cancers**

OX1R and OX2R mRNA was expressed in the rat glioma C6 cell line.

OXA significantly decreased cell viability of the rat glioma C6 cell line at a dose of 1 μM and resulted in an IC50 of 4.7 nM. OXB resulted in a non-statistically significant decrease in cell viability of the rat glioma C6 cell line.

**Orexin receptor expression in nerve tissue cancers**

OX1R mRNA was not expressed in the SK-N-MC cell line.

**Orexin receptor expression in chronic myelogenous leukemia**

OX1R and OX2R mRNA was expressed in chronic myelogenous leukemia (CML) clinical samples (Tables 2 and 3).

**Discussion**

**Orexin receptor expression in colon cancers**

Colorectal cancer samples comprise the most common study material for orexin treatment. It has been consistently demonstrated that both orexins A and B significantly induce apoptosis and inhibit growth of human colorectal adenocarcinoma (HT-29 and HT29-D4) cell lines. In mRNA and immunohistochemistry (IHC) studies, OX1R was observed in many colon cancer cell lines, namely HT-29, SW48, SW620, SW480, Caco-2, LoVo, Colo205, T84, LS174T, and in 38 clinical colon cancer samples. Surprisingly, OX1R mRNA was discovered in difficult-to-treat, 5-fluorouracil-resistant HT-29 cell samples (HT29-FU) as well as in 10 liver metastases of primary colon cancer samples. In addition, non-transformed, adjacent cells to the malignant clinical samples and metastases tested with quantitative real-time polymerase chain reaction (qRT-PCR) and IHC were negative for OX1R mRNA and staining.

OXA significantly decreased cell viability of the rat glioma C6 cell line at a dose of 1 μM and resulted in an IC50 of 4.7 nM. OXB resulted in a non-statistically significant decrease in cell viability in the rat glioma C6 cell line.

Cancers of the adrenal cortex. OX1R and OX2R mRNA was not expressed in rat pheochromocytoma (PC), PC12. OX2R mRNA was expressed in all human PC clinical samples tested, but OX1R mRNA was not.

OXA and OXB increased basal cortisol levels and proliferation in clinical samples of adenomas. OXA increased cortisol secretion, down-regulated OX2R mRNA, increased OX1R mRNA, and enhanced cell proliferation in the NCI-H295R cell line.

Cancers of the adrenal medulla. OX1R and OX2R mRNA was expressed on all clinical cortisol-secreting adenoma samples tested. OX1R and OX2R mRNA expression was variable for the NCI-H295R cell line.

OXA and OXB decreased tyrosine hydroxylase production in the PC12 cell line and increased inositol triphosphate (IP3), epinephrine, and norepinephrine release in clinical samples of PC.

**Orexin receptor expression in gastric cancers**

OXA treatment resulted in a protective effect in the gastric cancer cell line, BGC-823, including decreased caspase-3 apoptotic activity and improved cell proliferation and survival via the protein kinase B (AKT) signaling pathway. In this cell type, the presence of OXA appeared to lead to the overexpression of OX1R. Similar results were observed in the gastric cancer cell line, SGC-7901, which resulted in increased cell proliferation and viability and apoptosis.
Table 3. Orexin effects on cancer cell survival.

| System               | Organ     | Cell type     | OXA                                                                 | OXB                                                                 |
|----------------------|-----------|---------------|----------------------------------------------------------------------|----------------------------------------------------------------------|
| **Digestive system** | Colon     | HCT-116       | Induced autophagy (resistance to apoptosis), reduced cell viability, and promoted apoptosis; no significant effect | No significant effect                                               |
|                      |           | HT-29         | Induced significant apoptosis and inhibited cell growth              | Induced significant apoptosis and inhibited cell growth              |
|                      |           | HT29-FU       | Induced significant apoptosis                                        | Induced significant apoptosis                                      |
|                      |           | HT29-DR       | Suppression of cell growth and induction of apoptosis               | Suppression of cell growth and induction of apoptosis              |
|                      |           | SW48          | Induced significant apoptosis                                        | Induced significant apoptosis                                      |
|                      |           | SW620         | Induced significant apoptosis                                        | Induced significant apoptosis                                      |
|                      |           | SW480         | Induced significant apoptosis                                        | Reduced cell proliferation                                         |
|                      |           | Caco-2        | Induced significant apoptosis                                        | Reduced cell proliferation                                         |
|                      |           | LoVo          | Induced significant apoptosis                                        | Reduced cell proliferation                                         |
|                      |           | Colo205       | Induced significant apoptosis                                        | Reduced cell production                                             |
|                      |           | T84           | Induced significant apoptosis                                        | Reduced cell production                                             |
|                      |           | LS174T        | Induced significant apoptosis                                        | Reduced cell production                                             |
|                      |           | Xenograft LoVo | No effect                                                     | Reduced cell production                                             |
|                      |           | Xenograft HT29 | Drastic reduction in tumor size                                     | Reduced cell production                                             |
|                      |           | Xenograft HCT-116 | No effect                                                     | Reduced cell production                                             |
|                      | Stomach   | BGC-823       | Increased OX1R expression; 1.5-fold increase in cell proliferation and viability | Increased OX1R expression; 1.5-fold increase in cell proliferation and viability |
|                      |           | SGC-7901      | Stimulated cell proliferation and viability                          | Stimulated cell proliferation and viability                          |
|                      | Liver     | Hep3B         | Increased glucose uptake                                            |                                                                       |
| **Endocrine/ reproductive system** | Prostate | LNCaP         | Significantly decreased cell survival; significantly reduced androgen receptor nuclear translocation in the presence of testosterone | No effect                                                          |
|                      |           | DU145         | Increased cell growth in non-differentiated cells                   | Increased cell growth in non-differentiated cells                   |
|                      | Endometrium | ECC-1        | No apoptosis effect                                                | No apoptosis effect                                                  |
|                      |           | Ishikawa      | No apoptosis effect                                                | No apoptosis effect                                                  |
|                      |           | MFE-280       | No apoptosis effect                                                | No apoptosis effect                                                  |
|                      | Adrenal gland (cortex) | Clinical samples of adenomas | Increased basal cortisol levels; increased proliferation | Increased basal cortisol levels; increased proliferation |
|                      |           | NCI-H295R     | Increased cortisol secretion; down-regulated OX2R mRNA; increased OX1R mRNA; enhanced cell proliferation | Increased cortisol secretion; down-regulated OX2R mRNA; increased OX1R mRNA; enhanced cell proliferation |
|                      | Adrenal gland (medullary) | PC12 | Decreased tyrosine hydroxylase | Increased IP3, epinephrine and norepinephrine release |
|                      |           | Clinical samples of PC | Non-statistically significant decreased cell viability at a dose of 1 μM; IC50 of 4.7 nM | Non-statistically significant decreased cell viability |

OXA: orexin A; OXB: orexin B; PC: pheochromocytoma.

*Originally compiled summary of data from references cited within table.
protection via activation of the ERK1/2 pathway upon OXA treatment.\textsuperscript{13} OX1R, and not OX2R, was detected in both the BGC-823 and SGC-7901 cell lines tested.\textsuperscript{12,13} Therefore, in primary gastric cancers, orexin treatment may result in a counter-productive effect.

**Orexin receptor expression in hepatic cancers**

IHC and western blot analyses in the human hepatocellular carcinoma cell line, Hep3B, showed the presence of OX1R mRNA, but not OX2R.\textsuperscript{14} This finding was consistent in further studies of clinical human hepatocellular samples tested by IHC, which showed that 28 of 41 tumor samples and 9 of 14 non-tumor samples tested positive for OX1R, but were negative for OX2R.\textsuperscript{14} In addition, Hep3B cells incubated with OXA resulted in a concentration-dependent increased glucose uptake into the Hep3B cells, possibly promoting pyruvate shunting into the tricarboxylic acid (TCA) cycle and oxidative phosphorylation, and thereby reducing glycolysis.\textsuperscript{15} Further testing is required to determine whether this effect either promotes or decreases viability in this cell type.

**Orexin receptor expression in prostate cancers**

The results of mRNA testing for orexin receptor expression in prostate cancer cells are contradictory. Szychska et al.\textsuperscript{16} reported negative polymerase chain reaction (PCR) mRNA results for both OX1R and OX2R in normal prostate cell lines (PrEC, PrSc, PrSmC) and for prostatic carcinoma cell lines (DU145, LNCaP, and PC3). Alexandre et al.\textsuperscript{9} also observed undetectable OX1R mRNA in LNCaP cells. In contrast, Valiante et al.\textsuperscript{15} reported positive results for OX1R mRNA in the LNCaP cell line and that, upon treatment with OXA, LNCaP expression of OX1R increased while cell survival decreased.\textsuperscript{15} Noteworthy was the finding that OXA has an antagonistic effect in androgen receptor translocation in these cell types.

**Orexin receptor expression in cervical cancers**

IHC staining in clinical cervical cancer and cervicitis specimens were positive for expression of both OX1R and OX2R, but OX2R was found to be upregulated in 95.7% of cancer samples as opposed to 50.0% of cervicitis samples.\textsuperscript{17} No tests were completed to investigate the effect of orexin treatment in these cell types.

**Orexin receptor expression in endometrial cancers**

IHC testing results of clinical samples of endometrioid carcinomas (EEC) were negative for OX1R, while normal tissues tested positive for OX2R. This may be due to epigenetic silencing involving hypermethylation during the progression into cancer.\textsuperscript{18} OXA treatment in human endometrial cancer cell lines, ECC-1, Ishikawa, and MFE-280 did not result in apoptosis.\textsuperscript{18}

**Orexin receptor expression in adrenal cancers**

In normal tissues, the adrenal cortex (glomerulosa, fasciculate, and reticular zones) expresses OX1R, but not OX2R, while the adrenal medulla (epinephrine and norepinephrine cells) expresses only OX2R, but not OX1R.\textsuperscript{27}

**Cancers of the adrenal cortex.** Consistent with the normal distribution of orexin receptors in native tissues, Blanco et al.\textsuperscript{27} demonstrated by IHC that 4 clinical samples of adrenocortical adenomas tested positive for OX1R, while OX2R expression was absent. In another study of clinical samples, Spinazzi, et al.\textsuperscript{7} utilized qRT-PCR and western blot analysis methods to test 10 clinical adrenocortical adenoma tissues, which were all positive for the expression of both OX1R and OX2R mRNAs. In addition, it appeared that both receptors were overexpressed in adenomas as compared to normal adrenocortical tissue samples.\textsuperscript{7} When treated with orexins, OXA, alone, increased cortisol secretion, while both OXA and OXB treatment resulted in proliferative effects.\textsuperscript{7} This appears to reflect an upregulation of OX2R in malignancies versus normal tissues.

Studies of adrenal cortical cancer cell lines were also inconsistent. Wenzel et al.\textsuperscript{21} conducted a qRT-PCR analysis of National Cancer Institute (NCI) H295R human adrenocortical cells and reported high expression of OX2R. After treatment with OXA, the H295R cells resulted in significantly increased cortisol levels.\textsuperscript{21} It was also observed that OX2R receptor mRNA was down-regulated in response to OXA treatment. Although OX1R mRNA was present, it was found in very low levels.\textsuperscript{21} In contrast to the Wenzel et al.\textsuperscript{21} study, Chang et al.\textsuperscript{22} found that mRNA for OX1R, and not OX2R, was expressed in NCI-H295R (90%) prostate cancer samples, in 16 of 30 (53.3%) BPH samples, and in 8 of 30 (26.7%) chronic prostatitis samples.\textsuperscript{26} This may reflect an increased orexin receptor expression in malignant cells as a part of the transformational process of prostate cancer.
cells, expression of which was increased significantly subsequent to treatment of OXA, in a dose-dependent manner. Similar to Wenzel et al., Chang et al. found that OXA induced cortisol secretion from NCI-H295R cells, but additionally observed that cell proliferation was also enhanced. These inconsistent results demonstrate the complexity of the regulation of orexin receptors in the adrenal gland, especially in malignancies.

Cancers of the adrenal medulla. Studies of clinical samples of pheochromocytomas consistently report the presence of OX2R and an absence of OX1R, which is in line with endogenous tissue expression in these regions. Furthermore, OXA and OXB treatment of samples resulted in a 3-fold increase in IP3 production and a dose-related increase in both epinephrine and norepinephrine release.

In the Nanmoku et al.’s qRT-PCR study of the rat pheochromocytoma cancer cell line, PC12, an absence of both OX1R and OX2R expressions was observed. However, when the PC12 cells were treated with OXA and OXB, a dose-related reduction in tyrosine hydroxylase (the rate-limiting enzyme in catecholamine synthesis) was noted. Binding assays reflected that both OXA and OXB, in equal affinities, bound to the cells, which may indicate orexin interaction with a non-orexin receptor. This further adds to the complexity of orexin interaction with the adrenal glands.

Orexin receptor expression in brain cancers

In an experimental model of the aggressive brain cancer, glioblastoma multiforme (GBM), a qRT-PCR analysis was conducted with the rat C6 cell line, which confirmed the presence of both types of orexin receptors, similar to native tissue expression. Surprisingly, treatment of these cells with OXA, and not OXB, at a dose of 1 μM resulted in a significantly decreased rate of cell viability. The IC50 was calculated at 4.7 nM for OXA. However, lower (physiological level) doses of OXA did not compromise cell viability as compared to controls. Given this, orexin may provide a potential treatment option for this currently terminal condition.

Orexin receptor expression in nerve tissue cancers

Bader et al. completed a receptor study of the SK-N-MC neuroepithelioma cell line (controversially also regarded as a neuroblastoma cell line as it was originally derived from a metastatic neuroblastoma) and revealed an absence of OX1R at the mRNA level. In contrast, Rouet-Benigne et al. did not report mRNA findings for orexin receptor expression, but did complete a test-by-treatment study in the same cell line and showed that both OXA and OXB significantly inhibited cell growth (IC50 of 5 nM for both orexins) and induced apoptosis in SK-N-MC cells. Further testing is required to fully evaluate the orexin treatment potential in nerve tissue cancers.

Orexin receptor expression in CML

In a gene expression study of CD34+ hematopoietic stem and progenitor cells from newly diagnosed and untreated CML patients, a 1.8- to 2.2-fold upregulation of OX1R and OX2R was observed. Testing of this cell type with orexins to determine the effect of receptor stimulation may aid in the future analysis of orexin’s role in CML treatment, if any.

Study limitations

The authors note several study limitations. First, orexins are relatively recently discovered neurotransmitters, inherently limiting the volume of reviewable data available. In addition, the discovery of orexin-based cancer research began only in 2004, reducing the available period of time for completion of in vitro and in vivo studies.

Conclusion

Ectopic OX1R expression is common in many colon cancer cell lines (HT-29, HT29-D4, HT29-FU, SW48, SW620, SW480, Caco-2, LoVo, Colo205, T84, LS174T) and in clinical samples of localized and metastases of colon cancers. Significant induction of apoptosis or inhibited cell growth was observed in HT-29 and HT29-D4 cell lines upon treatment with orexins. In contrast, gastric cancer cell lines (BGC-823 and SGC-7901), while expressing OX1R, result in increased cell proliferation when treated with OXA. OX1R expression was shown in hepatic cancers (clinical hepatocellular tumor samples and Hep3B), but was not studied with orexin treatment for cell viability. While the results of prostate cancer cells were inconsistent, it appears that OX1R expression may be directly correlated to cancer progression. Furthermore, OX1R-positive prostate cancer samples treated with orexins results in reduced cell survival. It also appears that OX2R is upregulated in cervical cancer samples and down-regulated in endometrial cancer samples. The most common observations in cancers of the adrenal glands (clinical samples, NCI-H295R, PC12) show consistent orexin receptor expression that corresponds to non-transformed tissues in the region. Orexin treatment here resulted in changes in catecholamine production and, in some cases, increased cell proliferation. In rat C6 glioma cells (GBM model), high doses of OXA (1 μM) result in decreased cell viability (IC50 of 4.7 nM). In the neuroepithelioma cell line, SK-N-MC, one study showed a lack of orexin receptors, but another showed significantly inhibited cell growth (IC50 of 5 nM) upon treatment with OXA and OXB. Finally, clinical CD34+ CML samples resulted in a 1.8- to 2.2-fold up-regulation of OX1R and OX2R as compared to normal hematopoietic stem and progenitor cells. In whole, these findings demonstrate the complex, but intriguing role of orexin in the progression and treatment of various cancers.
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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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All of the authors have read and approved the paper for publication.

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