

Calcitriol Implications on Human Rhabdomyosarcoma: An Unexplored Field

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Abstract

This work is an extension of our published article in 2022 (<https://doi.org/10.1016/j.jsbmb.2022.106146>), where we further discuss any recent updates in this research and analyse the issues addressed in the focal article.

Calcitriol, the active form of vitamin D₃, is also acknowledged as 1 α , 25-dihydroxy vitamin D₃. This steroid hormone is known for its role in calcium homeostasis and bone metabolism. Recent studies suggest that calcitriol also exerts anti-cancer effects in various malignancies. We previously reported (in 2022) that treatment with this steroid hormone triggers apoptosis in RD cells, a known rhabdomyosarcoma (RMS) cell line. RMS is a common soft tissue sarcoma that develops malignant tumor derived from skeletal muscle cells, predominantly affecting children. However, high risk RMS treatment has not been improved over the last three decades, indicating that it is important to elucidate the molecular mechanisms underlying this disease. There is no updated information about the influence of calcitriol in RMS that could complement and extend our previously published article. However, we recognize the potential of this steroid hormone as a future therapeutic agent in managing this challenging cancer.

Rhabdomyosarcoma

Overview: past and newer subtypes

RMS is a malignant tumor that arises from skeletal muscle progenitor cells and is the most common soft tissue sarcoma in children [1] but also seen in adolescents and adults. Despite advancements in treatment, RMS remains challenging due to its aggressive nature and variable response to conventional therapies. Calcitriol, the hormonally active form of vitamin D, has been investigated for its potential anti-cancer properties [2]. This article supplements foregoing investigations [3] and examines how calcitriol affects RMS cells, focusing on its mechanisms of action and therapeutic potential. RMS was classified into several subtypes based on histological and genetic features [4]. The most common subtypes of RMS include embryonal rhabdomyosarcoma, alveolar rhabdomyosarcoma, and pleomorphic rhabdomyosarcoma, each varying in clinical

presentation, prognosis, and treatment response [5,6]:

Embryonal rhabdomyosarcoma: Includes botryoid variant, anaplasia and malignant ectomesenchymoma [7]. These present remarkable resemblance to developing embryonic skeletal muscle. It is the most common soft tissue sarcoma occurring in younger children and is often associated with a more favorable prognosis [1].

Alveolar rhabdomyosarcoma: Characterized by distinct alveolar structures and commonly affects adolescents and young adults. It is linked to specific genetic alterations, such as the PAX3-FKHR fusion gene [8].

Pleomorphic rhabdomyosarcoma: The least common subtype usually found in adults and associated with a more aggressive clinical course [9,10].

A new subtype of RMS that was genetically characterized, called Spindle Cell/ Sclerosing rhabdomyosarcoma [7]. The tumors affect patients of all age groups and are localized mostly in craniofacial bones [11].

Current treatment strategies

RMS represents about 4.5% of all pediatric cancers [12] and 50% of soft tissue sarcomas in this age group [13]. The current treatment strategies of this sarcoma typically involve a multimodal approach, incorporating surgery, chemotherapy, and radiation therapy. The choice of therapy is influenced by the tumor's histological subtype, stage, and location.

Surgery: Surgical resection remains the cornerstone of treatment for localized RMS. Complete surgical excision is crucial for improving prognosis, though achieving negative margins can be challenging, particularly in cases where the tumor is located in anatomically complex regions [14].

Chemotherapy: Chemotherapy is a fundamental component of the treatment regimen for RMS, particularly in cases with non-localized disease. The most commonly used chemotherapeutic agents include vincristine, dactinomycin, and cyclophosphamide, which have been shown to improve survival rates in both localized and metastatic RMS [15]. Recent advancements in chemotherapy regimens, such as the incorporation of ifosfamide and etoposide, have further enhanced treatment efficacy [16].

Radiation therapy: Radiation therapy is employed for local control, particularly when surgical resection is incomplete or when the tumor is located in a site where surgery is impractical. The role of radiation therapy is well-documented, and recent studies emphasize the importance of optimizing dosage to balance efficacy and minimize long-term side effects [17].

Emerging treatments and research

Recent research has explored targeted therapies and immunotherapy as potential adjuncts to conventional treatment. Targeted therapies aimed at specific genetic mutations, such as those involving the PAX-FOXO1 fusion gene in alveolar RMS, show promise in preclinical studies [18]. Additionally, immune checkpoint inhibitors and chimeric antigen receptor (CAR) T-cell therapy are being investigated in clinical trials, offering potential new avenues for treatment, especially in refractory or relapsed cases [19]. Molecular research into RMS pathology has revealed a strong connection between genetic and epigenetic alterations and key cellular processes such as growth, proliferation, differentiation, and apoptosis. The discovery of PAX-FKHR fusion genes has redirected research efforts towards understanding not only the mechanisms behind chromosomal translocations but also how these oncogenic fusion proteins modify cell phenotypes and can be targeted for therapeutic purposes [20].

Calcitriol

1 α , 25-dihydroxyvitamin D₃ (calcitriol), the active form of vitamin D₃, plays a crucial role in maintaining calcium homeostasis and bone health. It is synthesized in the kidneys from its precursor, 25-hydroxyvitamin D, in response to parathyroid hormone (PTH) stimulation and low serum calcium levels [21]. Beyond its well-known effects on calcium and bone metabolism, calcitriol has garnered interest for its broader biological functions, including its impact on cell proliferation, differentiation, and immune modulation [22]. It functions primarily through the vitamin D receptor (VDR), a nuclear receptor that regulates gene expression and also exerts rapid responses [23]. Recent research highlights its role in modulating cell proliferation, apoptosis, and differentiation in various cancer types [24].

Generalities of the involvement of calcitriol in cancer

The mechanistic effects of calcitriol in the cancer biology were recently reviewed [25]. They have explored the complex interactions initiated by calcitriol in cells and *in vivo* models of various cancer types, addressing topics such as angiogenesis, regulation of kinase cascades, and inflammation. Notably, the dual nature of calcitriol, as both a suppressor of adaptive immunity and an inducer of innate immunity, makes it an intriguing candidate for treating and preventing inflammation-driven carcinogenic processes. Moreover, its potential to promote the resolution of chronic inflammation and infection warrants further investigations. To point, calcitriol influences cell proliferation and differentiation through its interaction with VDR in a lot of different types of cancer [26]. In various cancer cell lines, calcitriol has been shown to induce cell cycle arrest and apoptosis, suggesting potential therapeutic applications in oncology [27]. Of relevance, this hormone modulates the immune system by influencing the function of both innate and adaptive immune cells. It has been observed to suppress the proliferation of T lymphocytes and modulate cytokine production, which can be beneficial in conditions characterized by excessive inflammation, such as autoimmune diseases related to cancer [28].

Calcitriol-dependent mechanisms of action in rhabdomyosarcoma

There are not recent studies investigating the potential of calcitriol as a therapeutic agent in RMS. Uhmman *et al.* reported that calcitriol promotes differentiation and additionally inhibits proliferation of ERMS tumor cells *in vitro* and *in vivo* [29]. Nevertheless, in our model of ERMS (RD cells) we did not observe calcitriol dependent morphology signs of differentiation [3]. Furthermore, we demonstrated that 1 nM of calcitriol (during 72 h) caused apoptosis of RD cells. However, further studies are needed to elucidate the apoptosis pathways involved in calcitriol dependent apoptosis in RD cells and in other RMS cell lines.

Conclusions

On one hand, calcitriol was proposed as a potential anti-cancer agent [30] and on other hand, it was also recognized that further investigations are needed to elucidate the molecular mechanisms of antineoplastic activity and optimal clinical applications of this steroid hormone in cancer [31]. Many studies indicate that vitamin D compounds possess cancer prevention and treatment properties in both *in vitro* and *in vivo* cancer models. Unfortunately, there is still limited information regarding the role of calcitriol in the treatment of rhabdomyosarcoma. Epidemiological studies also note an association between low vitamin D levels and the occurrence of cancer, as well as unfavorable outcomes [32]. Given its ability to induce differentiation or apoptosis, inhibit tumor growth and modulate the immune response, calcitriol holds promise as a therapeutic agent in RMS; however more research is needed to reveal the specific role of calcitriol in RMS. It is also necessary to elucidate the actions of calcitriol as a preventive and adjunct treatment in different cancers, including RMS.

Closing Comments

Since 1996, only three scientific studies referring to the actions of calcitriol in RMS have been published: Shabahang *et al.*, 1996 [33]; Uhmman *et al.*, 2012 [29], and Irazoqui *et al.*, 2022 [3].

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