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Commentary

Exploring Cell Differentiation, Induced by Inhibition of Phospholipase C/Protein Kinase C Cascade, as a Potential Therapeutic Strategy against Rhabdomyosarcoma

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Commentary

Among the reasons of childhood mortality, cancer is the leading cause of death [1,2]. Of all paediatric cancers diagnosed, soft tissue sarcomas account for 7%, of which approximately 50% are rhabdomyosarcoma (RMS), with an incidence of 4.71 per million children and adolescents [3,4]. Rhabdomyosarcoma (RMS) is the most common soft-tissue tumour in children and adolescents. This aggressive childhood cancer originates in normal skeletal muscle from myogenic cells that have failed to fully differentiate [5]. RMS can occur anywhere in the body, originating from mesenchymal cells destined to differentiate into skeletal muscle cells [6-8]. Treatment includes surgical resection, chemotherapy and radiotherapy, combined or not, depending on the location and tumour size, and the presence of metastases, in the latter case clinical outcomes are unsatisfactory [9]. Currently, the need for new treatments is emphasized, aimed at minimizing the long-term impact of therapy and improving patient life quality. One particularly notable characteristc of cancer cells is the loss of molecular markers associated with the differentiated state. As cancer progresses, some cells appear to be in a de-differentiated state closer to early developmental stages, similar to that of normal stem cells, with increased potential for self-renewal [10]. In this context, the ability to induce cell differentiation of tumoral cells is particularly valuable, as it can provide an alternative approach to combat the uncontrolled growth and malignant behavior of undifferentiated cancer cells.

Muscle cell differentiation, defined by the transition to a mature muscle cell phenotype, emerges as a critical process to counteract uncontrolled tumour proliferation and increase the efficacy of therapeutic treatments.

It´s known that the mechanisms driving skeletal muscle formation include transcription factors called MRFs (myogenic regulatory factors) such as, MyoD (myogenic differentiation protein 1), Myf5 (myogenic factor 5), Myogenin and Myf6 (myogenic factor 6), which regulate skeletal myogenesis, in which pluripotent mesodermal precursor cells proliferate and differentiate into myofibres, elongated multinucleated mature skeletal muscle cells specialised for contraction [11,12]. The muscle differentiation markers MyoD and Myogenin are expressed in RMS cells, but inappropriately bind to their target genes, avoiding the differentiation into mature skeletal muscle [13,14].

The importance was demonstrated that modulation of the Phospholipase C/Protein kinase C (PLC/PKC) pathway is related to skeletal muscle differentiation, through regulation of transcription factors involved in myogenesis such as activated T-cell nuclear factor, C1, C2 and C3 [15-18].

Interestingly, a recent work from our laboratory, focusing on the embryonic subtype of rhabdomyosarcoma (eRMS), since is the most commonly occurring subtype [19], revealed that indole family compounds (staurosporine and bisindolylmaleimide) and neomycin sulphate induce myogenic differentiation of RD (established human tumour cell line derived from an eRMS) and of C2C12 cell lines, both derived from a mesenchymal lineage. Our laboratory has extensive experience using both cell lines. They represent an adequate experimental model; specifically, the use of the RD cell line to study the pathology of rhabdomyosarcoma at a molecular level has been supported by numerous investigations.

Previous studies have shown that in conditions with low concentration of horse serum [20] or serum-free medium [21], C2C12 cells differentiate towards a mature cell profile (aligned, elongated and multinucleated cells). We were able to observe the same effect in RD cell line. However, the novelty of our work is that under proliferative conditions (DMEM with 10% fetal bovine or horse serum), and in precense of PKC inhibitors (bisindolylmaleimide 1nM or staurosporine 0.05 mM) or of indirect inhibitors of the kinase, through PLC, (neomycin sulphate 6 mM) we induced cell differentiation in the RD and C2C12 lines. We observed elongation and fusion of the cells from mononucleated to multinucleated, with increased alignment and directional uniformity. In addition, by Real Time PCR we determined the relative mRNA levels of myosin heavy chain 1 (MHC1, a specific marker of skeletal muscle differentiation), MyoD (an early expression molecule in the differentiation of myoblasts to myotubes) [11] and Integrin-α9 (a transmembrane glycoprotein, highly expressed in RMS) [22], using RD and C2C12 cells in a proliferation medium aconditionated with staurosporine, bisindolylmaleimide or neomycin sulphate. The results demonstrated that the different treatments with the PLC/PKC modulators affect the gene expression; particularly MHC1 and MyoD [19]. Data indicated that MHC1 mRNA was significantly increased in RD and C2C12 cells in presence of modulators, being more signficative with neomycin sulphate. Likewise, MyoD gene expression was upregulated by PLC/PKC modulators; this effect being more evident in presence of bisindolylmaleimide and neomycin sulphate. The Integrin-α9 mRNA levels clearly decreased in C2C12 cells. However, the results showed greater variability in RD cells. Thus, we decided to evaluate the protein level of integrin-α9. The results suggested that the treatments with staurosporine, bisindolylmaleimide or neomycin sulphate induce a decrease in protein expression levels.

The data obtained are relevant since new anti-cancer therapeutic strategies called ‘differentiation therapies’ focus on the induction of tumour cell differentiation signalling pathways, with the aim of inhibiting uncontrolled tumour growth and the invasion and metastasis mechanisms [23]. A differentiated tumour cell acquires morphological and functional characteristics specific to normal mature cells. Specifically, in RMS cells, this differentiation leads to a skeletal muscle cell, elongated multinucleated myotubes with sarcomeric structures. RMS cells do not express genes encoding the contractile apparatus (myosin, troponins, tropomyosins, myomesins and actinins) [27].

In RMS tumor cells, the ability to complete differentiation is impaired [24-26]; for example, RMS cells fail to express genes that encode the contractile apparatus [27].

Although studies have focused on developing therapies capable of restoring muscle differentiation potential in RMS cells [28-31], not all of them include the management of the signaling cascade involved.

We observed cell differentiation to mature skeletal muscle fibre in RD and C2C12 cells cultured in both low serum and serum-free media. Then, we propose that the cells, under stress caused by the absence of certain essential growth factors, and with the consequent arrest of cell proliferation, are forced to adopt a developmental path different from that initially programmed. This state of cellular stress activates two possible response pathways: it could be apoptosis, or it could promote survival through a differentiated phenotype. In the latter case, the cell transitions from the active cell cycle to a metabolically less demanding state, analogous to the G0 phase of the cell cycle. This shift involves less energy consumption and a functional focus on cell specialisation, allowing the cell to adapt to adverse conditions while retaining its integrity.

Of importance for the bases of new therapies, we demonstrated that treatments with staurosporine, bisindolylmaleimide or neomycin sulphate in a proliferative medium induced RD and C2C12 cells to a mature muscle phenotype. Indeed, we observed in both cell lines, stretched and multinucleated fibres distributed in a uniform direction, coupled with an increased expression of myodifferentiation markers such as MHC1 and MyoD. In addition, and very relevant, the treatments induced a decrease in the expression levels of integrin-α9 (protein overexpressed in the more aggressive phenotypes of RMS cells).

The differentiation process involves the loss of the hallmarks that are essential for tumour establishment and progression [32]. Hallmarks include sustained activation of proliferative signalling pathways, the ability to evade inhibitory signals of cell growth, and the acquisition of mechanisms that facilitate invasion of healthy tissues. These properties, intrinsically linked to the malignant phenotype, represent pivotal points in the establishment and the aggressiveness of tumor cells.

The PKC pathway plays a key role in the regulation of cell differentiation through precise modulation of gene expression and interaction with multiple intracellular signalling cascades [33]. These cascades, in turn, influence processes essential for cellular architecture and functionality, including the reorganisation of the cytoskeleton, the dynamics of intercellular adhesions and the remodelling of the extracellular environment. PKC acts as an integrating node that coordinates biochemical and mechanistic signals, adjusting the transcriptional machinery and signalling networks to the specific needs of the differentiated state, underlining its relevance in the maintenance of homeostasis and in the adaptive responses of cells to external stimuli.

PLC modulates signalling cascades that, among other actions, control intracellular calcium release, and PKC activation. In addition, PLC is involved in the regulation of gene expression, the establishment of cell-cell connections and the interaction with the extracellular microenvironment. Its role as a signal integrator allows it to act as an essential regulator in physiological processes such as cell proliferation, differentiation and migration [34].

Based on the results obtained, we propose that the PLC/PKC-mediated signalling pathway could play a significant inhibitory role in the process of cell differentiation. This effect was evidenced by using both direct (staurosporine and bisindolylmaleimide) and indirect (neomycin sulphate) inhibitors of PKC kinase, resulting in an increased commitment of the RD and C2C12 cell lines towards a differentiated phenotype. This finding suggests that modulation of PKC activity alters critical signalling pathways involved in differentiation, possibly by regulating transcription factors involved in myogenic process.

These results are promising in the development of cell differentiation-based therapeutic strategies specific for eRMS. Further studies are required to unravel precisely the molecular mechanisms and specificity of the observed effects, including detailed analysis of the interactions between PLC/PKC signalling pathways and other potential mediators.

In conclusion, our work provides insight into the molecular mechanisms regulating cell differentiation in C2C12 and RD cell lines, the latter being representative of embryonal rhabdomyosarcoma. Our research highlights staurosporine, bisindolylmaleimide and neomycin sulphate as molecules capable of inducing muscle cell differentiation through inhibition of PLC/PKC-mediated signalling pathways, even under proliferative culture conditions. These results underline the importance of understanding the mechanisms involved in cell differentiation and their modulation as a strategy to reverse the malignant phenotype, opening new possibilities for the design of therapeutic interventions aimed at addressing tumour resistance and improving clinical outcomes in patients with embryonal rhabdomyosarcoma.

Conflicts of Interest

All the authors declare that they have no conflict of interest.

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