Phospholipase D Induced Differentiation in MG63 Osteoblast-like Cells in Response to Surface Energy Involves Protein Kinase C

Mimi Fang¹, Marco Wieland², Barbara D. Boyan¹, and Zvi Schwartz¹

Georgia Institute of Technology¹ and Institut Straumann AG, Basel, Switzerland²

Statement of Purpose: It has been established that biomaterial surface properties such as chemical composition, topography, and energy can change cellular responses at the cell-implant interface (1). Recently, it has been shown that surface roughness activates phospholipase D (PLD) activity in osteoblast-like MG63 cells to mediate differentiation (2). There are two different isoforms of PLD, PLD1 and PLD2. These isoforms share ~50% homology, but they are regulated and localized differently in the cell. PLD1 is activated by protein kinase C (PKC) and G-proteins such as Rho and Arf, whereas PLD2 is not (3). The main function of PLD is to hydrolyze membrane phosphatidylcholine to generate the precursor signaling molecule phosphatidic acid (PA) and choline. PA can be metabolized into diacylglycerol (DAG), which can activate PKC. PKC has been shown to regulate many short and long term signaling events such as receptor desensitization, cell adhesion, membrane transport, cell motility, and cell differentiation (4). We hypothesize that osteoblast differentiation on rough and high energy titanium (Ti) surfaces is mediated through a PLD-dependent pathway involving increased PKC activity.

Methods: Human osteoblast-like MG63 cells were cultured on three types of pure Ti surfaces (Institut Straumann AG, Basel, Switzerland) and tissue culture polystyrene (TCP). The three Ti surfaces used were smooth pretreatment surfaces (PT), acid-etched rough surfaces (SLA), and SLA surfaces modified to have a higher surface energy (modSLA). When cells were confluent on TCP, one half of cultures on all surfaces were treated for 24 hours with the PLD inhibitor, ethanol (EtOH). Conditioned media were collected and assayed for osteocalcin (OCN) and osteoprotegrin (OPG). Cell layers lysates were assayed for total protein content, alkaline phosphatase, PLD, and PKC activity. In separate cultures, RNA was extracted for RT-PCR of PLD1 and PLD2.

Results: RT-PCR showed that the cells expressed mRNAs for both PLD1 and PLD2 (data not shown). PLD activity increased as the surface roughness and energy increased (Fig 1A). Cell number decreased (data not shown) and OCN (Fig 1B) and OPG (not shown) increased as surface roughness and energy increased. PLD and PKC activity were increased on modSLA (Fig 2A, B), corresponding to increased alkaline phosphatase (Fig 2C) and OCN (Fig 2D). EtOH (0.01%) blocked the surface effect on these parameters.

Conclusions: In general, the higher surface energy Ti surface (modSLA) caused a significantly larger cell response than the other surfaces, thus showing the importance of surface chemistry. Surface roughness and energy induced PLD activity was positively correlated with osteoblast differentiation. Moreover, inhibition of PLD blocked this effect, indicating a role for PLD in the process. PLD may also have a role in osteoclast maturation, since PLD inhibition decreased the amount of OPG produced by the osteoblasts. Furthermore, we showed that PKC is regulated by surface roughness and energy induced PLD, suggesting that PKC is downstream from PLD, and indicating that PLD2 is the main isoform involved in this pathway. In the future, to further confirm the main PLD isoform involved, we plan on using short interfering ribonucleic acids to knock down the expression of each isoform. We will also be investigating whether PLD is activated by the hormone 24R,25 dihydroxy-vitamin D₃ similarly to the pathway in resting zone chondrocytes (5).


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