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ESTUDO DA INTERAÇÃO ENTRE PrP^C E STI1/HOP NA BIOLOGIA DE CÉLULAS-TRONCO DE GLIOBLASTOMA HUMANO *IN VIVO*

Tese apresentada ao Departamento de Biologia Celular e do Desenvolvimento, do Instituto de Ciências Biomédicas da Universidade de São Paulo, para a obtenção do Título de Doutor em Ciências.

Área de Concentração: Biologia Celular e Tecidual

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RESUMO

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O glioblastoma é o tipo mais comum e agressivo de glioma, um tumor de Sistema Nervoso Central (SNC) formado por células gliais que apresenta 100% de letalidade. Dados da literatura apontam para uma subpopulação tumoral que apresenta características de células-tronco, chamadas células-tronco de glioblastoma (CTGs), que seriam responsáveis pela proliferação, invasão, resistência à terapia, angiogênese e recidiva tumoral. Portanto, a elucidação dos mecanismos que governam a biologia destas células é essencial para o desenvolvimento de terapias mais eficazes contra o GBM. Neste estudo visamos identificar o papel das proteínas prion celular (PrP^C), uma glicoproteína ancorada à membrana plasmatica e seu ligante, *stress inducible protein 1* ou *heat shock organizing protein* (STI1/HOP), uma co-chaperona abundantemente secretada por células do SNC, na manutenção da proliferação e autorrenovação de CTGs. Linhagens de glioblastoma humano U87 e U251 cultivadas como neuroesferas e enriquecidas de células-tronco foram utilizadas como modelo de estudo. Populações expressando baixos níveis de PrP^C e/ou HOP, através de *knockdown* por sistema lentiviral ou *knockout* por CRISPR/Cas9, foram utilizadas para compreensão da função destas proteínas na biologia de células-tronco. Nossos resultados demonstram que o silenciamento de PrP^C é capaz de reduzir a expressão de marcadores de células-tronco como Sox2 e CD133 e inibir a autorrenovação celular, indicando PrP^C como uma molécula chave para a manutenção do estado indiferenciado de CTGs. Além disso, observamos co-localização e co-expressão de PrP^C e CD133 na superfície celular, sendo a internalização de CD133 estimulada com íons cobre e associada a PrP^C, sugerindo a modulação da expressão de CD133 na membrana plasmática por PrP^C. Adicionalmente, o silenciamento de PrP^C diminui a expressão de proteínas de adesão, como E-caderina e α6-integrina, e afeta diretamente a migração celular, implicando PrP^C em processos de invasão tumoral. Interessantemente, o peptídeo de HOP que mimetiza o sítio de interação a PrP^C ($pepHOP_{230-245}$) é capaz de bloquear a formação do complexo na superfície e inibir a proliferação e autorrenovação mediada por HOP em células positivas para PrP^C. Por sua vez, o silenciamento de HOP reduz a proliferação celular, a qual pode ser recuperada com o tratamento com HOP recombinante em células que expressam PrP^C, indicando um papel importante deste complexo na proliferação de CTGs. Observamos que a tumorigenicidade de neuroesferas expressando baixos níveis de PrP^C e/ou HOP é significativamente reduzida, bem como a capacidade proliferativa destas células *in vivo*, indicando o complexo PrP^C-HOP como potencial alvo para o desenvolvimento de novas terapias com base no controle da proliferação de CTGs.

Palavras-chave: Glioblastoma, Células-tronco de glioblastoma, Proteína prion celular, Heat shock organizing protein, Proliferação, Autorrenovação.

ABSTRACT

IGLESIA, R. P. **Role of PrP^C and STI1/HOP in human glioblastoma stem cells biology *in vivo*.** 2017. 130 p. Ph. D. Thesis (Cell and Tissue Biology) - Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2017.

Glioblastoma is the most common and aggressive type of glioma, Central Nervous System tumor formed by glial cells that presents 100% lethality. Literature data suggest that a tumor subpopulation, stem cells-like called glioblastoma stem cells (GSCs), may be responsible for proliferation, invasion, resistance to therapy, angiogenesis and tumor recurrence. Therefore, elucidation of the mechanisms that govern the biology of GSCs is essential to develop more effective therapies against GBM. In this study we aimed to identify the role of cellular prion protein (PrP^C), a membrane-anchored glycoprotein and its ligand, stress inducible protein 1 or heat shock organizing protein (STI1/HOP), a co-chaperone protein, in the maintenance of cell proliferation and self-renewal of GSCs. Glioblastoma U87 and U251cell lineages cultured as neurospheres were used as model to study tumor stem cell biology. Populations expressing low levels of PrP^C and/or HOP, through knockdown by lentiviral system or knockout by CRISPR/Cas9, were used to identify the function of these proteins in stem cell biology. Our results demonstrate that PrP^C silencing is able to reduce the expression of stem cell markers such as Sox2 and CD133 and to inhibit cellular self-renewal, indicating PrP^C as a key molecule for the maintenance of the undifferentiated state of GSCs. In addition, we observed co-localization and co-expression of PrP^C and CD133 on cell surface, and CD133 internalization stimulated by copper ions associated with PrP^C, suggesting the modulation of CD133 expression on the cell surface by PrP^C. Additionally, PrP^C silencing decreases the expression of adhesion proteins, such as E-cadherin and α6-integrin, and directly affects cell migration, implying PrP^C in tumor invasion processes. Interestingly, the HOP peptide which mimics PrP^C binding site ($pepHOP_{230-245}$) is able to inhibit the complex formation on the cell surface and the proliferation and self-renewal mediated by HOP in PrP^C-positive cells. On the other hand, HOP silencing decreases cell proliferation, which in turn can be recovered by treatment with recombinant HOP in cells expressing PrP^C, indicating an important role of this complex in the proliferation of GSCs. We observed that the tumorigenic ability of neurospheres expressing low levels of PrP^C and/or HOP is significantly reduced, as well as the proliferative capacity *in vivo*, revealing the PrP^C-HOP complex as a potential target for the development of new therapies based on the control of GSCs proliferation.

Keywords: Glioblastoma, Glioblastoma stem cells, Cellular prion protein, Heat shock organizing protein, Proliferation, Self-renewal.

Introdução

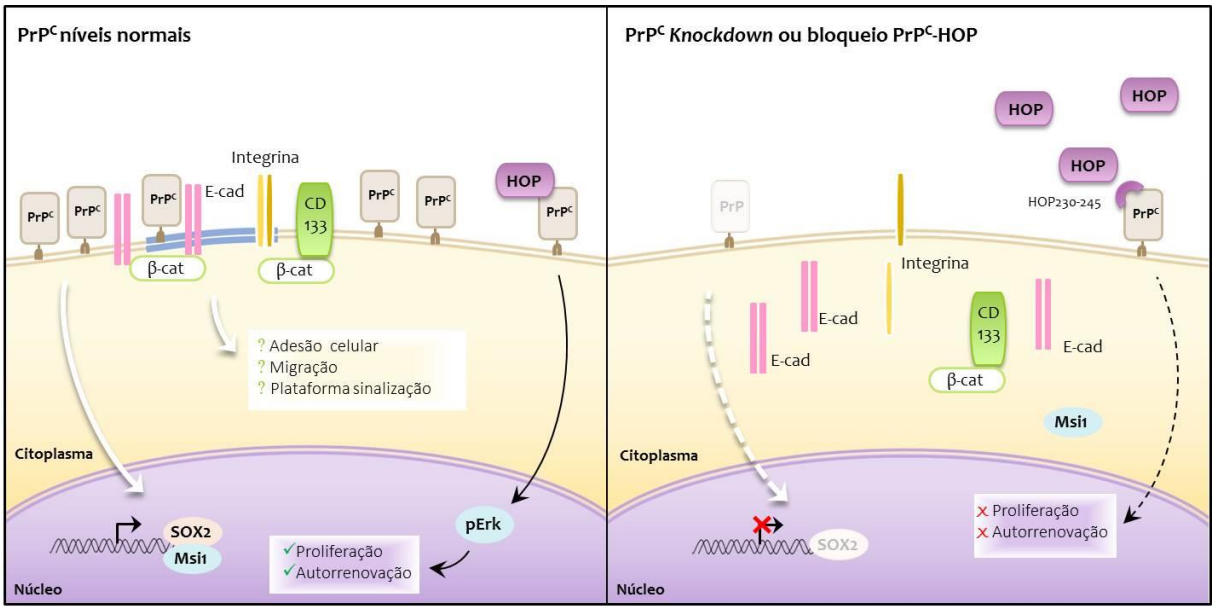
O glioblastoma (GBM) é um tumor de sistema nervoso central (SNC) extremamente agressivo e por apresentar 100% de letalidade faz se necessário o estudo aprofundado dos mecanismos que regulam sua manutenção. A proposta deste estudo foi avaliar o papel da proteína prion celular (PrP^C), uma glicoproteína de superfície celular abundantemente expressa no SNC e que pode funcionar como *scaffold protein*, ou seja, modulando diversas funções biológicas através da interação com diferentes ligantes, na biologia de células-tronco de glioblastoma (CTGs). A interação entre PrP^C e um de seus principais ligantes, *stress inducible protein one* ou *heat shock organizing protein* (STI1/HOP), foi especialmente investigada devido ao papel deste complexo na proliferação de células-tronco neurais (CTNs), estas semelhantes a CTGs, e de glioblastomas cuja manutenção é regulada pelas CTGs.

Neste contexto, avaliamos os efeitos da perda-de-função de PrP^C e HOP em GBM humano, bem como o bloqueio da interação entre estas proteínas com um peptídeo de HOP que mimetiza a interação com PrP^C, visando identificar novas moléculas alvo para o desenvolvimento de terapias mais eficazes contra o GBM.

Conclusão

Devido à agressividade do GBM, estudos que visam o desenvolvimento de novas estratégias terapêuticas contra esse tipo de tumor estão em curso. Em particular, CTGs têm sido consideradas alvos em potencial, uma vez que estas células têm características bem estabelecidas nos tumores. O esquema 7 resume nossos achados, demonstramos que na presença PrP^C (painel esquerdo) moléculas de adesão como Integrina e E-caderina estão ativas na superfície, bem como CD133 que é recrutado para a membrana plasmática e marcadores de células-tronco SOX2 e Musashi-1 são expressos no núcleo. HOP interage com PrP^C modulando a proliferação celular através da ativação da via de Erk1/2. Com a dimunição da expressão de PrP^C na superfície (painel direito), moléculas de adesão, CD133 e Musashi-1 permanecem no citoplasma e a expressão de SOX2 no núcleo está ausente. A proliferação e autorrenovação mediadas por HOP recombinante não ocorrem pela ausência de PrP^C na superfície e através do bloqueio da interação PrP^C-HOP pelo peptídeo $pepHOP_{230-245}$. Em conjunto, estes resultados indicam PrP^C como um possível indicador de prognóstico, uma vez que a presença de células-tronco está diretamente relacionada a malignidade do GBM, além de apresentar novas moléculas alvo para o desenvolvimento de terapias contra o GBM visando a inibição da proliferação de CTGs.

Esquema 7 – Resumo dos dados do estudo.



Esquema 7 - Desenho esquemático das conclusões do estudo.

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