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CARACTERIZAÇÃO *IN SILICO* DO MODELO DE INTERAÇÃO DE GENES DA AUTO-RENOVAÇÃO DAS CÉLULAS TRONCO E SINALIZAÇÃO POR CÁLCIO EM METÁSTASES DE MELANOMA HUMANO

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Caracterização *in silico* do Modelo de Interação de Genes da Auto-Renovação das Células Tronco e Sinalização por Cálcio em Metástases de Melanoma Humano

Trabalho de Conclusão de Curso submetido à Universidade Federal do Rio Grande do Sul como parte dos requisitos necessários para obtenção do Grau de Bacharel em Biotecnologia.

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RESUMO

Melanoma é um câncer de pele muito suscetível à ocorrência de metástases e, por isso, é considerado uma neoplasia maligna agressiva. Até hoje, mais de 90% das causas de morte por câncer são atribuídas à ocorrência de metástases. Acredita-se, ainda, que especificamente um subconjunto de células da população de um tecido tumoral é capaz de semear e repopular o seu tumor de origem. Estudos evidenciam que essas células "iniciadoras" de tumores comumente possuem características semelhantes às de células tronco, tais como poder de auto-renovação e capacidade de dar origem a células progenitoras ou diferenciadas, o que lhes rendeu o nome de células tronco tumorais (CTTs). Existem evidencias de que a via de sinalização do íon cálcio tem papel fundamental em uma série ampla de processos biológicos, dentre os quais estão o comprometimento celular na diferenciação de células tronco. Sendo assim, nós questionamos no presente estudo a influência da via de sinalização do Ca^{2+} na regulação da pluripotência de células tronco dentro de um contexto de neoplasia maligna. Para isso, nós criamos uma rede *in silico* ("STEMCa") de interação proteína-proteína para modelar a integração de ambas as vias (sinalização por Ca^{2+} e regulação de pluripotência de células tronco). Após, nós avaliamos a expressão dos genes que codificam as proteínas desse modelo, utilizando um repositório público de microarranjos. Para os dados de expressão, foram utilizadas apenas amostras clínicas de melanomas metastáticos (tendo tumores primários como controle) de três conjuntos independentes de amostras de pacientes. Por fim, utilizamos ferramentas de biologia de sistemas para inferir potenciais candidatos a alvos terapêuticos e/ou marcadores de metástase. Nossa análise de expressão diferencial indica que, em ao menos um dos conjuntos de amostras de pacientes, 51% dos genes do modelo construído (151 de um total de 294 genes) encontram-se consistentemente desregulados (valor de *p* corrigido <0.05) em metástases de melanoma quando são comparados com amostras de tumor primário, sugerindo que, de fato, as vias avaliadas têm expressão aberrante. Além disso, nossos cálculos topológicos em cima do modelo proposto apontam *GNAQ*, *GSK3B*, *GSTP1*, *MAPK3*, *PPP1CC*, *PRKACA*, e *SMAD4* como potenciais candidatos a serem estudados para intervenções terapêuticas ou como biomarcadores.

Palavras-chave: célula tronco tumoral; metástase; cálcio; pluripotência; biologia de sistemas.

ABSTRACT

Melanoma is a skin cancer with high incidence to develop metastasis and, therefore, it is considered to be an aggressive malignant neoplasm. To date, more than 90% of cancer-related causes of death are still attributed to the occurrence of metastasis. It has been postulated that a subset of cells within the tumor tissue is solely capable of seeding and restore the whole tumoral tissue. A number of studies have reported that these tumor-initiating cells also named as *cancer stem cells* (CSCs) commonly display stem-like properties, such as self-renewal and also potency (*i.e.* they are able to give rise to progenitor or differentiated cells). Calcium ion (Ca^{2+}) signaling pathway plays a fundamental role on a broad series of biological processes, and its role in stem cell fate and commitment to differentiation has additionally been proposed. Hence, one could speculate whether Ca^{2+} signaling pathway and stem cell pluripotency regulation crosstalk may influence the metastatic transformation of melanoma cancer cells. In order to find evidence for this role, we build an *in silico* protein-protein interaction network ("STEMCa") to model the integration of both pathways (Ca^{2+} signaling and stem cell pluripotency regulation). Thereafter, we evaluated the expression pattern of the genes coding for the proteins within the "STEMCa" model by utilizing three publicly available microarray datasets from human patients found in Gene Expression Omnibus (GEO). For our analysis, we compared data from clinical samples of melanomas metastasis with primary tumors (controls). Finally, we used systems biology tools to identify putative therapeutic targets *in silico* and/or candidate biomarkers for melanoma metastasis. Our analysis revealed that the expression profile of the genes within our "STEMCa" model is consistently deregulated, with 151 out of 294 of its members (51%) displaying significant changes of expression (corrected *p*-value <0.05) in at least one of the datasets of melanoma metastases, when compared with primary biopsy tumors. This suggests that the integrated pathways comprising our network are aberrant in gene expression. In addition, topological measurements of "STEMCa" model point towards *GNAQ*, *GSK3B*, *GSTP1*, *MAPK3*, *PPP1CC*, *PRKACA*, and *SMAD4* as optimal candidates *in silico* to be further studied as potentially good therapeutic targets or biomarkers.

Key words: cancer stem cell; metastasis. calcium; pluripotency; systems biology

SUMÁRIO

1. Introdução

Fundamentalmente todo organismo multicelular origina-se de uma única célula com o potencial de dar origem a todas as linhagens celulares que compõe os seus tecidos (Dreesen e Brivanlou, 2007). Tal célula deverá apresentar duas propriedades básicas, as quais os biólogos denominam:

- Auto-renovação: por divisão celular assimétrica, uma célula tronco (CT) divide-se em duas células, de maneira que uma das células é idêntica a célula mãe (*i.e.* com as mesmas propriedades de uma CT), e a outra compromete-se com algum destino celular e detendo algum grau menor de características de CT.
- Potência: é a capacidade que uma célula possui de dar origem às novas linhagens celulares. Em humanos, por exemplo, faz-se a seguinte classificação: a célula é totipotente se for capaz de originar todos os tipos celulares do organismo; pluripotente se for capaz de originar qualquer célula das três camadas germinativas (mas não do trofoblasto extraembrionário) ou, por fim, multipotente se for capaz de dar origem a um conjunto restrito de células tecido-específicas dentro de uma única camada germinativa. Nessa classificação, ainda podemos citar as células oligopotentes (capazes de dar origem a um conjunto mais restrito ainda de tipos celulares em comparação às multipotentes) e também as unipotentes (capazes de gerar apenas células do próprio tipo).

Embora o papel das células tronco (CTs) seja evidente no desenvolvimento embrionário, os tecidos adultos mantém uma pequena subpopulação de CTs adultas que mantém as propriedades de auto-renovação e pluri ou multipotência, com a finalidade de manter e reaver a homeostase dos tecidos em caso de lesões (Brack e Rando, 2012).

Conceitualmente, células tronco tumorais (CTTs) possuem características semelhantes às de CTs, tais como poder de auto-renovação e capacidade de diferenciarem-se em novos tipos celulares, porém apresentam perda do controle do ciclo celular (característica tumoral) (Allan *et al.*, 2006). Devido a tais características, discute-se, nas últimas décadas, a hipótese de que na heterogenea população tumoral existe uma pequena subpopulação de CTTs que são capazes de sozinhas semear a tumorigênese. De fato, já em 1937, Furth *et. al.*

reportaram que apenas uma pequena porção de células era capaz de induzir tumorigênese em camundongos após esses animais terem recebido injeção subcutânea de poucas células oriundas de um tumor leucêmico (Furth e Kahn, 1937). Contudo, foi apenas em meados dos anos 90 que a hipótese das CTTs como semente dos tumores ganhou força, especialmente através dos trabalhos de Dick *et. al.*. Esses autores separaram células $CD34^{+}/CD38^{-}$ (raras na população heterogênea do tumor) de leucemia aguda mielóide e as transplantaram para comundongos imunodeficientes, resultando na mesma doença e com características semelhantes às do tumor que a originou, ao passo que outras células testadas não apresentaram este desfecho (Lapidot *et al.*, 1994). Ou seja, supostamente um pequeno subconjunto de toda população tumoral é suficiente para repopular todo o tumor de origem. Vale ressaltar, sobretudo, que CTTs já foram também identificadas em tumores sólidos, tais como câncer de mama (Al-Hajj *et al.*, 2003), cérebro (Singh, Hawkins, *et al.*, 2004), pulmão (Eramo *et al.*, 2008) e pele (Schatton *et al.*, 2008).

Acredita-se que as CTTs podem se originar de CTs adultas normais por aquisição de mutações em oncogenes, fazendo com que a célula mutada perca o controle da regulação do ciclo celular e passe a dividir-se de maneira aberrante (Li *et al.*, 2007). De fato, muitas proteínas de superfície marcadoras de CTs adultas são também encontradas em CTTs, sugerindo uma relação de origem entre ambas. Outra hipótese é a de que as CTTs originam-se de células tumorais diferenciadas, mas que adquiriram mutações que induziram-nas a um processo de desdiferenciação, fazendo-as adquirir características de CTs (Li *et al.*, 2007). Sabe-se que esse tipo de reprogramação celular é factível, pois já foi demonstrado que é possível transformar fibroblastos humanos em células pluripotentes através da indução da expressão ectópica de alguns fatores de transcrição nessas células (Park *et al.*, 2008). No entanto, a regulação das vias que controlam essa reprogramação celular *in vivo* são muito complexas, de maneira que torna-se conceitualmente difícil dar crédito ao surgimento espontâneo de CTTs a partir de células diferenciadas. Por outro lado, tendo em vista que a proporção de CTs para as demais células de um tecido qualquer é extremamente baixa, isso torna a ideia do acúmulo de mutações em CTs também um cenário improvável. Todavia é importante lembrar que o estado de diferenciação celular não é uma propriedade discreta, portanto existe uma gradiente de células com fenótipos intermediários em abundância relativa nos tecidos. Além disso, nenhuma dessas hipóteses é mutuamente exclusiva, de maneira que é possível que ocorram concomitantemente.

As CTTs já foram também implicadas na recorrência de tumores pós-tratamento, devido sua característica intrínseca de resistência aos quimioterápicos, os quais tem como estratégia terapêutica clássica a indução da morte celular por apoptose (Zhao, 2016). As próprias CTs normais já apresentam altos níveis de expressão de genes relacionados à sobrevivência e anti-apoptóticos, ou seja, possivelmente as CTTs contem com sistemas de proteção pré-existentes (He *et al.*, 2014). Além disso, a superexpressão de bombas de efluxo multidrogas ATP-dependentes já foi reportada para CTTs de vários tumores, o que demonstrou-se apresentar alta correlação com os níveis de reincidência de câncer (Koren e Fuchs, 2016). Vale ressaltar ainda que as CTTs comumente apresentam altos níveis de expressão de aldeído-desidrogenase (ALDH), o que também tem sido correlacionado ao poder de invasividade e quimioresistência (Croker *et al.*, 2009). Por exemplo, Chafaret-Jaufret *et al* demonstraram que células $ALDH^+$ isoladas de câncer de mama são mais invasivas que as $ALDH^-$ (Charafe-Jauffret *et al.*, 2010). E, ainda em câncer de mama, Kai *et* al apontam que encontra-se preferencialmente uma subpopulação $CD44^+/CD24^-$ (marcador tronco) em tecidos metastáticos oriundos de amostras clínicas (Kai *et al.*, 2011).

Outro evento molecular que tem sido vinculado a metástases em alguns tipos de câncer é a presença de oscilações intracelulares de cálcio (e.g., câncer de mama e de próstata) (Rizaner et al., 2016). Em geral, O íon cálcio (Ca^{2+}) tem papel importante nos sistemas biológicos por suas propriedades químicas intrínsecas, sendo este um íon divalente com mais afinidade a átomos de oxigênio (O) do que nitrogênio (N) ou enxofre (S) (Carafoli e Krebs, 2016). Além disso, o Ca^{2+} é capaz de ligar-se a todos os átomos de O de grupamentos carboxilados de biomoléculas. Tais propriedades marcam esse íon como uma espécie bastante reativa em sistemas biológicos, e os organismos evoluíram de modo a utilizá-lo como agente sinalizador, provavelmente pela elevada exposição ao mineral cálcio (abundante na crosta terrestre). Por outro lado, essa alta reatividade implicou também a evolução de mecanismos para controlar a concentração intracelular de Ca^{2+}, tais como:

- Compartimentalização: o Ca^{2+} é depositado e mantido em diferentes subcompartimentos celulares.
- Externalização/Internalização: o Ca^{2+} pode ser bombeado através das membranas dos subcompartimentos celulares quando necessário.
- Quelação: biomoléculas ligam-se estavelmente ao Ca^{2+}, aprisionando-o e impedindo que se ligue a outras moléculas.

A ligação do Ca^{2+} a biomoléculas pode tanto ativá-las quanto reprimí-las, pois as interações eletrostáticas induzem alterações conformacionais nas proteínas, alterando sua estrutura tridimensional e, portanto, sua funcionalidade. Em geral, a sinalização por cálcio refere-se a um sinal induzido ou transduzido pelo Ca^{2+} por meio da elevação de sua concentração intracelular, disparando uma série de reações e, por fim, re-estabilizando os níveis de Ca^{2+} no estado basal. A dinâmica do sinal é tal que o Ca^{2+} é sequestrado e trocado entre subcompartimentos, aqui incluídos citosol, mitocôndria, retículo endoplasmático, cisternas trans do complexo de Golgi e núcleo (Berridge *et al.*, 2003). A sinalização por Ca^{2+} tem papel fundamental em diversos processos biológicos, tais como excitabilidade, exocitose, mobilidade, apoptose, transcrição e diferenciação — para citar alguns (Clapham, 2007). O que distingue um sinal de outro é essencialmente a amplitude e a frequência de variação da concentração intracelular de Ca^{2+}, além da natureza do próprio estímulo. Sendo assim, as oscilações de $[Ca^{2+}]$ na célula são finamente reguladas por uma maquinaria celular que, quando corrompida, pode levar a uma série de patologias, incluindo a transformação, promoção e progressão de câncer (Tsai *et al.*, 2015).

De entre todos os tipos de câncer, o melanoma é um dos mais suscetíveis ao desenvolvimento de metástases e, portanto, muito agressivo. Segundo estimativas do Instituto Nacional do Câncer (INCA), o melanoma representa, no Brasil, cerca de 3% das neoplasias malignas. Embora a incidência seja baixa, a letalidade desse tipo de câncer é bastante elevada e suas chances de cura são baseadas principalmente na detecção precoce do tumor. Alguns marcadores correlacionados a CTTs já foram associados ao poder metastático de melanomas, tais como CD20, CD133, CD271 (três proteínas de superfície), ABCB5 (membro da família das bombas de efluxo ATP-dependentes) e ALDH1A (uma isoforma da ALDH) (Kozovska *et al.*, 2016). Com referencia ao cálcio, tem sido recentemente descrito a super-expressão de canais de cálcio tipo T em melanoma humano sendo estes vinculados à proliferação celular (Maiques *et al.*, 2016). Além disso, a concentração de cálcio no soro humano correlaciona com o estágio do melanoma cutâneo, sendo este sugerido como marcador da progressão do câncer (Datta *et al.*, 2016). Contudo, o papel do cálcio e de moléculas dependentes da sinalização por cálcio no processo de metástases é pouco compreendido e tem sido muito menos estudado.

No intuito de conectar esse aparato de informações, surge o interesse em se estudar o papel da integração das vias de sinalização do Ca^{2+} concomitantemente à regulação da pluripotência de CTs no contexto de metástase em melanomas humanos. Para atingir o

objetivo de se estudar vias tão complexas simultaneamente e de maneira eficaz, torna-se indispensável a utilização de métodos computacionais. A Biologia de Sistemas é uma área da Bioinformática que aborda a modelagem de redes de interação entre biomoléculas e/ou metabólitos e compostos através de grafos. Na matemática, a Teoria de Grafos vem sendo desenvolvida desde o século XVIII, e atualmente são muitos os métodos para se analisar suas propriedades (mesmo para grafos de elevada complexidade). Sendo assim, pesquisadores vêm usufruindo desses métodos para melhor entender o perfil do funcionamento modular das redes proteicas em células, haja visto que um organismo vivo opera sob influência conjunta de milhares de biomoléculas interagindo entre si. Atualmente, são muitos os bancos de dados com coleções de anotações para todo tipo de informação biológica (seja para genômica, proteômica, metabolômica, interatômica etc), viabilizando o processo de modelagem de sistemas biológicos. No estudo a seguir, montamos uma rede de interações proteína-proteína, integrando as vias de sinalização do cálcio e regulação da pluripotência de células tronco, e avaliamos a dinâmica de expressão dos genes que codificam essas proteínas em diferentes amostras primárias de melanomas humanos, comparando o perfil de expressão de tumores primários e metastáticos.

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Network-based Identification of Altered Stem Cell Pluripotency and Calcium Signaling Pathways in Metastatic Melanomas

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Running title: Aberrant stem cell-Ca^{+2} signaling network in melanoma metastases.

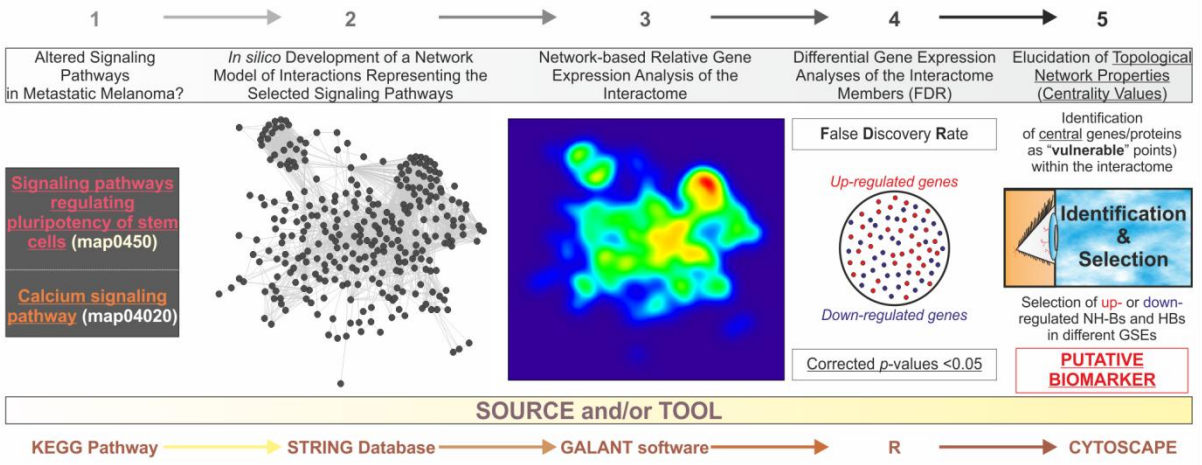
Abbreviations: CSC, cancer stem cell; CON, connector; FDR, false discovery rate; HB, hub- bottleneck; NH-B, non-hub-bottleneck; PPI, protein-protein interaction; SC, stem cell.

ABSTRACT

Malignancy of cancer has been linked to distinct subsets of stem-like cells, the so-called "cancer stem cells" (CSCs) which persist during treatment and seems to lead to drug-resistant recurrence. Metastatic spread of cancer cells is one of the hallmarks of malignancy and contributes to the majority of human melanoma-related deaths. Overlapping groups of proteins and pathways have recently been identified to regulate both stem cell migration and cancer metastasis, raising the question whether genes/proteins involved in stem cell pluripotency may have important implications when applied to the biology of cancer metastasis. Furthermore, it is well known that ion channels and receptors, particularly those responsible for calcium (Ca^{2+}) signal generation, are critical in determining the cellular fate of stem cells (SCs). In the present study, we searched for evidence for altered stem cell pluripotency and Ca^{2+} signaling-related genes in the context of melanoma metastases when compared to primary tumors, by network analysis of gene expression of biopsy tissue from three different datasets of patients. We created an *in silico* network model ("STEMCa" interactome) showing the landscape of interactions between stem cell pluripotency and Ca^{2+} signaling-related genes/proteins and demonstrated that around 51% (151 out of 294) of the genes within this model displayed significant changes of expression (FDR, corrected *p*-value <0.05) in at least one of the datasets of melanoma metastases when compared with primary biopsy tumors. Analysis of the topological network properties (*degree* and *betweenness*) revealed 27 members as the most central hub- (HB) and non-hub-bottlenecks (NH-B) among the 294 genes/proteins of the whole interactome. From those representative genes, *CTNNB1*, *GNAQ*, *GSK3B*, *GSTP1*, *MAPK3*, *PPP1CC*, *PRKACA*, and *SMAD4* showed equal up- or down-regulation (corrected *p*-value <0.05) in at least 2 datasets of melanoma metastases samples and only *PTPN11* showed up-regulation (corrected *p*-value <0.05) in the three of them, when compared with primary tumor samples. We postulate that altered expression of stem cell pluripotency and Ca^{2+} signaling pathways-related genes may contribute to the metastatic transformation, being these central members an optimal candidate group of biomarkers and *in silico* therapeutic targets for melanoma metastasis.

Keywords: pluripotency; stemness; calcium; malignancy; biomarker; systems biology.

Graphical Abstract



Description. General abstract workflow summarizing the criteria and tools utilized for the present study. Two maps from the KEGG PATHWAY Database corresponding to "Signaling pathways regulating pluripotency of stem cells" (map04550) and "Ca^{2+} signaling pathway" (map04020) were selected. Thereafter, an interactome was developed for integrating these maps into one single gene/protein interaction network model by the STRING V10.0 database (http://string-db.org/). With GALANT, we projected numerical data from three different datasets extracted from GEO database (http://www.ncbi.nlm.nih.gov/geo/). The 2D views correspond to the relative gene expression onto the newly-developed interactome. This tool creates three smoothed data plots that resemble the network model layout. Differential gene expression analysis was performed by using the R environment (*limma* package). The *p*-values were adjusted for multiple comparisons by calculating false discovery rates (FDR), in order to avoid potential false positives. By using Cytoscape, further identification of "vulnerable" points within the *in silico* model upon elucidation of centrality values and selection of those central (NH-Bs and HBs) up- or down-regulated genes, suggest candidate diagnostic biomarkers and also potential molecular targets for therapeutics.

1. Introduction

During the last 20 years of cancer research, the arise of the so-called cancer stem cell (CSC) theory still generates controversies among the scientific community as critical players in tumorigenesis, with the main characteristics of (**i**) initiate, (**ii**) maintain, (**iii**) re-grow diverse tumors and (**iv**) to give rise to drug-resistant recurrence [1,2]. The molecular mechanisms of cancer metastases are still under study but they are known to develop from small populations of cancer cells within a primary tumor, capable of entering and survive in the circulation and then exit to re-grow in a distant tissue [3]. The way the CSC theory may fit with the general scheme of metastatic potential is not well-defined to date.

Melanoma is a neoplasm stemming from melanocytes or the cells that develop from melanocytes, and since the increase in popularity of the CSC theory, single stem cells markers have been reported in the literature to correlate with melanoma malignancy, such as ABCB5, CD20, CD133, CD271, and ALDH1A [4]. These studies invite for further investigation about the potential role o stem cell pluripotency-related genes/proteins in its progression and potential metastatic transformation. Considering that stem cells (SCs) have the capability of self-renew and to differentiate into more specialized cells [5], the signaling pathways governing their properties are also subject of intense research. For instance, ion channels and its receptors, particularly, those responsible for calcium (Ca^{2+}) signal generation, are critical for determining cellular fate and differentiation of SCs and transcriptional changes in members of Ca^{2+} signaling pathway may thus trigger pathological events (*e.g.*, tumorigenesis) [6,7]. The possible link between intracellular Ca^{2+} and metastasis of human cancer cells is still poorly comprehended. A recent study (2016) by Rizaner and colleagues observed spontaneous Ca^{2+} oscillations in a proportion of strongly metastatic human prostate and breast cancer cells [8].

Probably, the biggest challenge for cancer researchers resides on the discovery of robust markers for prognosis and metastasis, in order to provide early diagnoses and treatment of patients.

The detection and follow-up of disease markers is nowadays based on solitary proteins, and this approach is most of the times unreliable. Wider and more powerful systems than conventional pathology-based data (e.g., immunohistochemical), capable of dealing and analyze increased amount of information are needed such as bioinformatics and systems biology tools [9]. For example, by the integration of additional information in biological pathways or a protein-protein interaction (PPI) network models, we could find "modular biomarkers" that integrate multiple genes with potential interactions that lead to more accurate and reproducible prognostic predictions and better comprehension of the disease pathogenesis [10]. In fact, our group has successfully applied network-based approaches and systems biology analyses in the context of diverse pathologies such as autism, Alzheimer´s disease, and periodontal inflammation [11-17].

In this study, we created an integrated *in silico* network model of interactions in order to seek evidence for altered stem cell pluripotency and Ca^{+2} signaling pathways in microarray samples from three independent datasets of patients. By using a system biology-based approach, we search for de-regulated central genes at the transcriptional level that may represent putative biomarkers for melanoma metastases. Based on our findings, we hypothesize that aberrant signaling of stem cell pluripotency and Ca^{+2} pathways may account for metastatic transformation, and specifically propose 9 representative central genes of our whole interactomic model as optimal candidate group of biomarkers for human metastatic melanoma.

2. Material and methods

2.1 Development of the gene/protein interaction network model, data acquisition and processing

In order to build our integrated *in silico* network model (“STEMCa”), we first retrieved all genes annotated as members of the KEGG PATHWAY Database entries x and y, which are respectively “Signaling pathways regulating pluripotency of stem cells” (map04550) and “Ca^{2+} signaling pathway”

(map04020) (**Supplementary table 1**). The human interactome (*i.e.*, annotated interactions between human proteins) was downloaded from STRING V10.0 database (http://string-db.org/) [18] in a text file and read into the R programming environment, where scripts were written to generate our PPI network. We only used interactions reported by STRING as being from either "database" or "experimental evidence" and also with a minimum score of 0.400 (medium confidence). The basic steps for network generation were as follows: (**i**) proteins encoded by the KEGG gene list were used as input to build the PPI network with the set parameters; (**ii**) the algorithm searches for disconnected nodes on the network (*i.e.*, proteins with no reported interaction with the main network); (**iii**) if there are disconnected nodes, the algorithm will hunt for neighbor proteins that could be included into the network, or "connectors" (CONs) with the purpose of joining disconnected nodes to the main network; (**iv**) if two or more nodes are able to join a set of disconnected nodes to the main network, then the one with the highest degree is selected; and finally, (**v**) the algorithm only stops when there are no more nodes that can be added. The rationale behind this approach is to build a fully connected PPI network model from a list of protein with the least interference possible from *outsiders* (proteins that were not originally inputs) without losing network density connection.

2.2 Relative gene expression network visualization and microarray analysis

For our expression meta-analysis, we searched for cancer microarray raw data on GEO database (http://www.ncbi.nlm.nih.gov/geo/) by using as keywords: "melanoma" and "metastasis" or "metastatic melanoma" in *Homo sapiens*. Selected GSEs were GSE8401 (N=83), GSE46517 (N=104), GSE15605 (N=58). The database is publicly available and data were originally contributed by Xu et al. (2008) [19], Kabbarah et al. (2010) [20], and Raskin et al. (2013) [21], respectively. Experimental assays are explained in detail in these original publications; including the selection criteria of patients. Raw data was normalized by the use of robust array normalization (RMA). Differential gene expression analysis was performed through the implemented functions for R on the *limma* package,

which uses linear models to calculate moderated t-statistics for microarray data. The *p*-values were adjusted for multiple comparisons by calculating false discovery rates (FDR) [22]. Only genes reporting corrected *p*-values <0.05 (FDR) were considered to be differentially expressed (**Supplementary table 2**). Log fold changes for each of the encoding genes of the proteins on our *in silico* model were exported from R into text files and plotted over the PPI network (metastatic vs. primary melanoma samples) by using the pluggin GALANT (GrAph LANdscape VisualizaTion) for Cytoscape (gaussian function sigma set to 0.04), which builds functional landscapes onto biological networks [23].

2.3 Elucidation of the topological network properties (degree or connectivity and betweenness)

Elucidation of the topological network properties [24] such as *degree* (also known as *connectivity)* and *betweenness* centralities (**Supplementary table 2**) was performed by using the Network Analyzer plugin from the Cytoscape software. Values of centralities above one standard deviation (+1SD) of the mean were selected to identify central nodes within the interactome. *Degree* gives information about the local topology of each node by summing up the number of its adjacent nodes. Nodes with high values of *degree* over the thresholds values are known as ‘‘hubs’’. *Betweenness* provides values about how frequently the shortest path, connecting every pair of nodes, is going through a third given node. Therefore, *betweenness* provides information about the influence of a node over the spread of information throughout the interactome. Nodes with values of *betweenness* over the thresholds are named as ‘‘bottlenecks’’ (non-hub and hub-bottlenecks are represented by NH-Bs and HBs, respectively).

3. Results

3.1 "STEMCa" is an integrative network model of interactions for stem cell pluripotency and Ca^{+2} signaling pathways

Two pathways, the stem cell pluripotency (map04550) and Ca^{2+} signaling (map04020) (**Supplementary table 1**) are integrated within the interactome (**Fig. 1**), being composed of 294 nodes interconnecting through 2995 interactions. In the case of non-existing direct crosstalk through common members from both signaling pathways, it is possible to search for common neighboring nodes corresponding to additional genes/proteins or CONs to members of both pathways by the use of mathematical algorithms in the R environment (see "*Material and methods*" section). Our model included 21 CON within the "STEMCa" interactome.

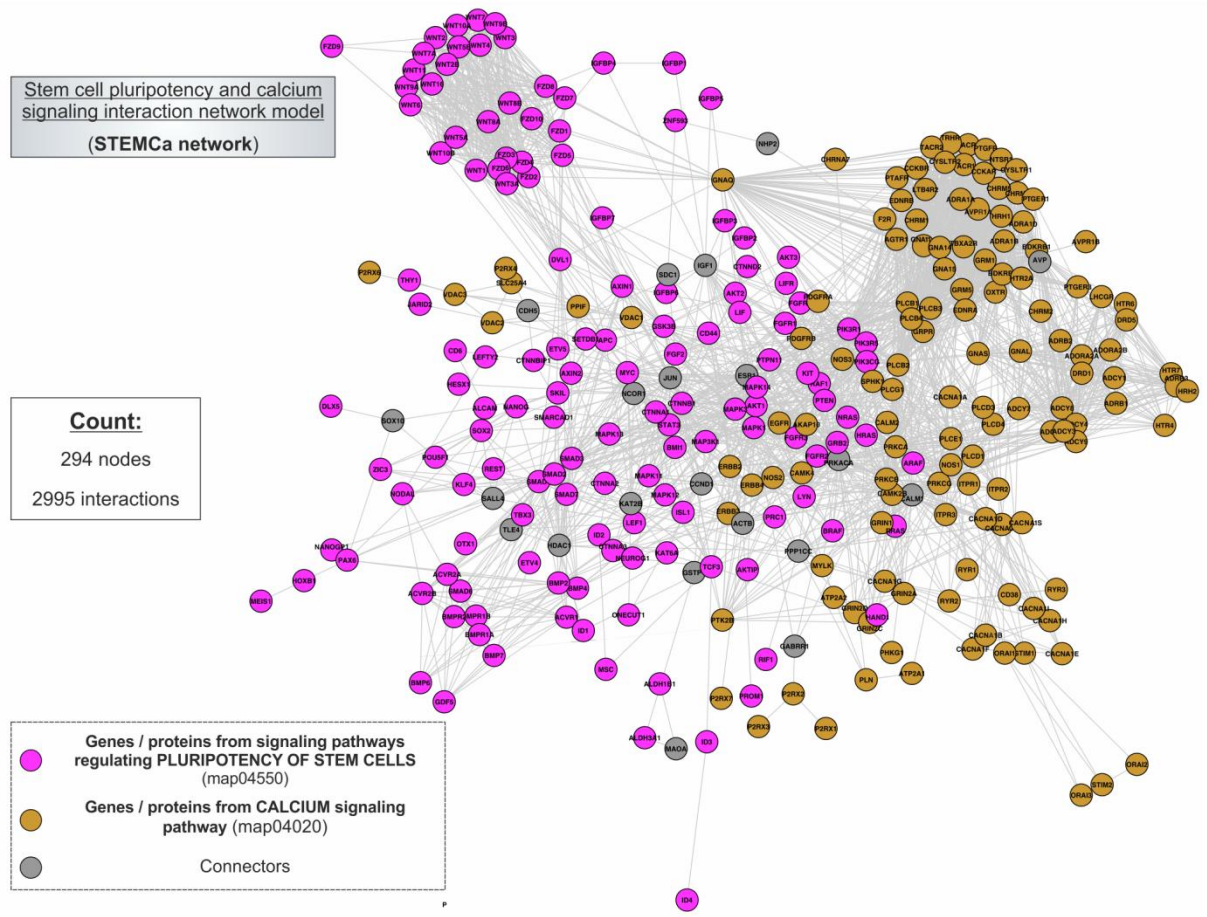


Figure 1. "STEMca" interactome. General landscape of interactions between genes/proteins of stem cell pluripotency (map04550) and calcium signaling (map04020) interaction network model. The present *in silico* network model was developed by using the STRING V10.0 database resource search tool, exclusively using ''Experiments'' and ''Databases'' as input options for active prediction methods and a minimum confidence score of 0.400 (medium confidence).

3.2 Network-based analyses of stem cell pluripotency and Ca^{+2} signaling pathways reveal transcriptional changes in independent datasets of human metastatic melanoma

We performed a systems level analysis of potential transcriptional changes in genes belonging to SC pluripotency and Ca^{+2} signaling pathways in metastatic melanomas. The "STEMCa" interactome (**Fig. 2A**) was subjected to relative gene expression analysis profile by using the GALANT software, which plotted the expression mean values derived from metastatic melanoma over the expression of primary tumor samples in the network model, presenting it as two dimensional GALANT-generated landscapes graded by colors (**Fig. 2B-D**). The relative gene expression levels are color-coded with warm colors (yellow-red) being representative of higher expression, whereas cold (green-blue) colors represent lower gene expression, when compared to control samples.

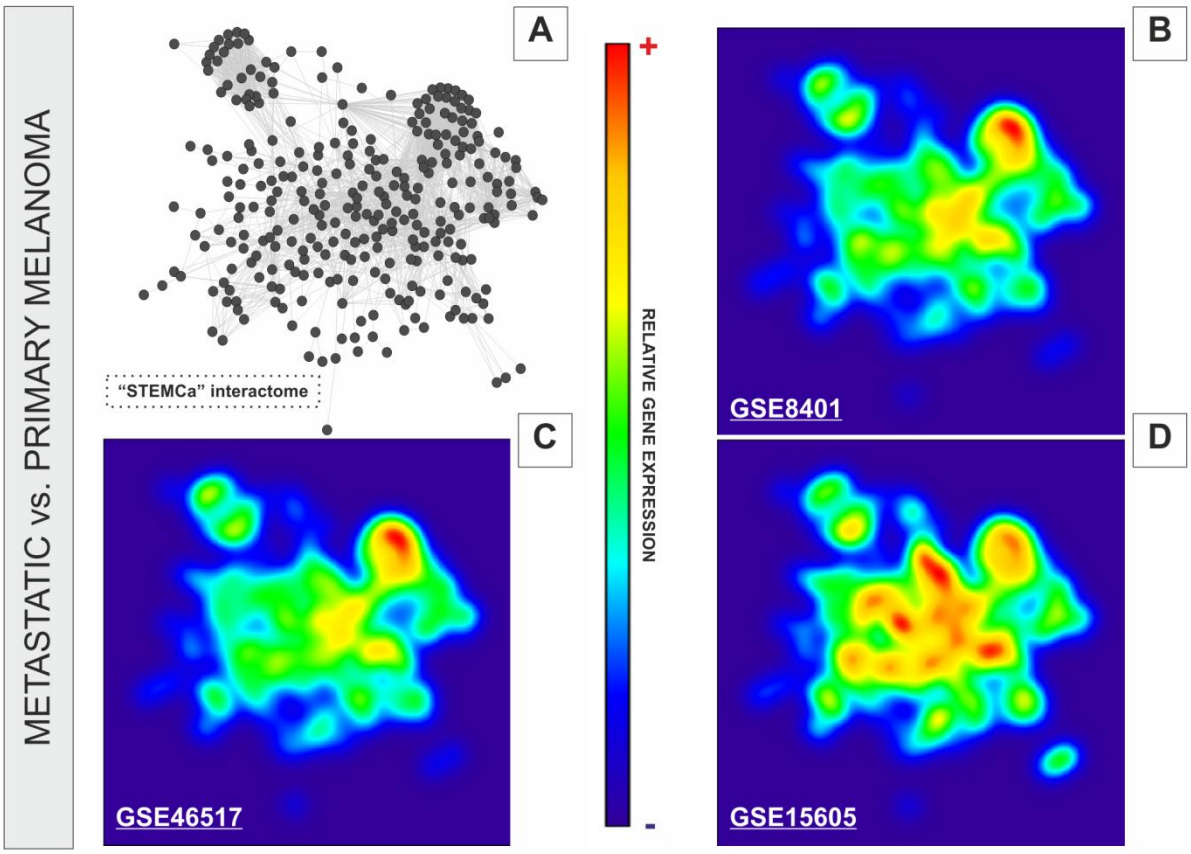


Figure 2. GALANT plots over the *in silico* model "STEMca" in melanoma metastases from three independent sample sources from GEO. The figure represents the relative gene expression over the "STEMca" interactome (A) in melanoma metastases vs. primary melanoma analyzed in (B) GSE8401, (C) GSE46517, and (D) GSE15605 independent micro-array samples, by using the GALANT software which is an open-source application able to build landscape maps of gene expression networks.

The 2D GALANT plots revealed equal landscape pattern of de-regulated relative gene expression in SC pluripotency and Ca^{+2} signaling pathways when metastatic melanomas were compared to primary tumors in three independent datasets (GSE8401, GSE46517, and GSE15605) of microarray samples (**Fig. 2B-D**). Differential expression analysis of "STEMCa" interactome revealed significant transcriptional changes in metastatic melanoma. In particular, 151 (~51%), 63 (~22%), and 14 (~5%) out of 294 genes from "STEMCa" interatome are differentially expressed (corrected *p*-values <0.05; FDR) in these tissue samples from one, two, or three independent microarray datasets, respectively (**Supplementary table 2**). At this stringent FDR, none of these changes were expected to be a false-positive. Of note, in all cases these genes follow the same pattern of de-regulation (up- or down-regulation) when datasets are compared (**Supplementary table 2** and **fig. 2**). The observed changes may account for the metastatic transformation of human melanoma with consequent alterations in patient survival.

3.3 Elucidation of key hub genes/proteins within the integrative "STEMCa" interactome suggest a group of candidate biomarkers for metastatic melanoma

For characterizing the newly-developed interactome, we calculated the topological network properties or centrality values (**Supplementary table 2**), in particular *degree* or *connectivity* and *betweeness*. These values reveal the most central nodes (genes/proteins) in a network [14-16]. Central nodes (NH-B and HB) mark points of "vulnerability" within the network of interactions and could consider that any variations in these members (*e.g.*, up- or down- regulation of given gene) can trigger more intense changes in the rest of the members of the interactome (*e.g.*, "STEMCa") than those in non- or less central nodes. Therefore, one could consider that any gene/protein identified as NH-B or HB may represent one putative biomarker [16], especially if those showed the same qualitative up- or down-regulation in gene expression (corrected *p*-values <0.05; FDR), validated in at least two independent datasets of microarray samples. After calculating centrality values for the 294

"STEMCa" gene/protein members (**Supplementary table 2**), we identified 27 central nodes (**Fig. 3**), 19 NH-Bs (values of *betweenness* above one standard deviation of the mean) and 8 HBs (values of *betweenness* and *degree* above one standard deviation of the mean).

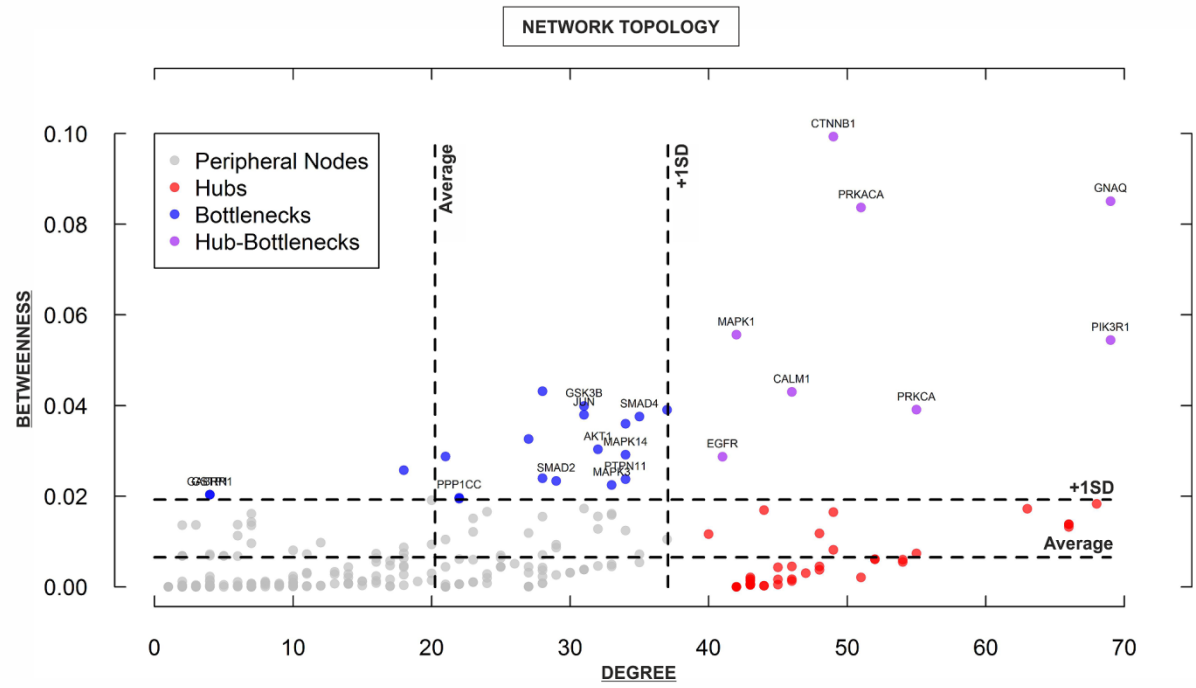


Figure 3. Analysis of the topological properties (*betweenness* vs. *degree*) of genes/proteins belonging to the "STEMca" interactome. The figure identifies only central nodes, hub-bottlenecks (HBs) and non-hub-bottlenecks (NH-Bs) with values above +1SD of the mean, which are differentially expressed (corrected *p*-values <0.05) in at least one GSE from the re-analyzed cohorts (GSE8401, GSE46517, and GSE15605).

Respectively, 11, 5, and 1 gene/s out of 19 NH-Bs showed same qualitative (up- or down-regulation) differential gene expression (corrected *p*-values <0.05; FDR) in these tissue samples from one, two, or even three independent microarray datasets. 8 and 3 out of 8 HBs showed same qualitative differential gene expression (corrected *p*-values <0.05; FDR) in 1 or 2 GSEs datasets (**Table 1**). In particular, *CTNNB1*, *GNAQ*, *GSK3B*, *GSTP1*, *MAPK3*, *PPP1CC*, *PRKACA*, and *SMAD4* genes (corrected *p*-value <0.05; FDR) are over- or sub-expressed in at least 2 datasets of melanoma metastases when compared with primary tumor samples. Exclusively *PTPN11* or protein tyrosine phosphatase, non-receptor type 11 showed up-regulation (corrected *p*-value <0.05; FDR) in the three re-analyzed cohorts of patients (**Table 1**).

GENE INFORMATION			TOPOLOGY			GSE8401		GSE46517		GSE15605	
Gene Symbol	**Ensemble ID**	**Sub.**	**Degree**	**Betweeness**	**NH-B HB**	**Diff. Exp.**	**Corrected *p*- value** (FDR)	**Diff. Exp.**	**Corrected *p*- value** (FDR)	**Diff. Exp.**	**Corrected *p*- value** (FDR)
AKT1	ENSP00000270202	ST	32	**0.03034247**	NH-B	---	0.073094859	**Down**	**0.00257 2769**	---	0.99159187
CALM1	ENSP00000349467	CON	**46**	**0.04297522**	HB	---	0.420640218	**Up**	**0.01435 4422**	---	0.102753445
CTNNB1	ENSP00000344456	ST	**49**	**0.09934346**	HB	**Up**	**0.0001128 83**	**Up**	**5.22113 E-05**	---	0.134736908
EGFR	ENSP00000275493	Ca	**41**	**0.02867496**	HB	---	0.454469806	**Down**	**4.09354 E-18**	---	0.372953188
ESR1	ENSP00000206249	CON	37	**0.03902762**	NH-B	---	0.343618 01	---	0.69191 3409	---	0.858356832
GABRR1	ENSP00000412673	CON	4	**0.02033756**	NH-B	---	0.516750581	---	0.17880 7962	**Up**	**0.0302361 48**
GNAQ	ENSP00000286548	Ca	**69**	**0.08508797**	HB	**Up**	**0.0147697 85**	**Up**	**0.03812 2837**	---	0.174777485
GSK3B	ENSP00000324806	ST	31	**0.03985954**	NH-B	**Down**	**4.43231E-06**	**Down**	**2.75596 E-10**	---	0.542667881
GSTP1	ENSP00000381607	CON	4	**0.02033756**	NH-B	**Down**	**3.14081E-05**	**Down**	**2.61693 E-06**	---	0.274434846
HDAC1	ENSP00000362649	CON	27	**0.03262084**	NH-B	---	0.213434449	---	0.71099 9269	---	0.865172009
IGF1	ENSP00000302665	CON	28	**0.04312205**	NH-B	---	0.697266013	---	0.43146 5367	---	0.669567 75
ITPR3	ENSP00000363435	Ca	22	**0.01942364**	NH-B	---	0.269623007	---	0.67187 5922	---	0.742562621
JUN	ENSP00000360266	CON	31	**0.03792962**	NH-B	**Down**	**8.92807E-06**	---	0.17136 6276	---	0.900656682
MAPK1	ENSP00000215832	ST	**42**	**0.05561423**	HB	**Up**	**0.0004121 72**	---	0.42909 1242	---	0.608460569
MAPK14	ENSP00000229794	ST	34	**0.02912488**	NH-B	---	0.605738821	**Up**	**0.03864 2425**	---	0.358766287
MAPK3	ENSP00000263025	ST	33	**0.02244545**	NH-B	**Down**	**1.10052E-05**	**Down**	**0.01074 679**	---	0.999319565
MYC	ENSP00000367207	ST	34	**0.03598441**	NH-B	---	0.602836201	---	0.27405 7104	---	0.449615647
NCOR1	ENSP00000268712	CON	21	**0.02875081**	NH-B	---	0.627351808	---	0.25784 9878	---	0.148831132
PIK3R1	ENSP00000274335	ST	**69**	**0.05441236**	HB	---	0.383906559	**Down**	**0.03005 9149**	---	0.057825554
PPP1CC	ENSP00000335084	CON	22	**0.01965509**	NH-B	**Up**	**1.52233E-05**	**Up**	**3.22551 E-05**	---	0.307997432
PRKACA	ENSP00000309591	CON	**51**	**0.0837037**	HB	**Down**	**0.0019389 8**	**Down**	**0.00616 6758**	---	0.056947292
PRKCA	ENSP00000408695	Ca	**55**	**0.03906664**	HB	**Up**	**0.0038609 94**	---	0.22782 6787	---	0.934220679
PTPN11	ENSP00000340944	ST	34	**0.02374219**	NH-B	**Up**	**0.0399083 43**	**Up**	**0.00909 2992**	**Up**	**0.0482640 37**
SMAD2	ENSP00000262160	ST	29	**0.02336588**	NH-B	**Up**	**0.0094664 33**	---	0.92471 9732	---	0.23296179
SMAD3	ENSP00000332973	ST	28	**0.02392531**	NH-B	---	0.975647588	---	0.09794 078	---	0.219862918
SMAD4	ENSP00000341551	ST	35	**0.03753624**	NH-B	**Up**	**5.56315E-06**	**Up**	**0.00029 2094**	---	0.141417242
TCF3	ENSP00000262965	ST	18	**0.02573384**	NH-B	---	0.803481248	---	0.21713 9092	---	0.260191349
MEAN			20.37414	6.56E-03							
SD			16.80553	0.01276070							
MEAN +1SD			37.17968	0.01932203							

Table 1. Centrality values (*degree* and *betweenness*) for each node (gene/protein) within the stem cell pluripotency (map04550) and calcium signaling (map04020) interaction network model ("STEMCa" network). Differentially expressed genes within these members are listed. Centralities over the thresholds with value/s above one (+1SD) standard deviation of the mean are color-marked. Corrected *p*-values <0.05 were considered significant for differentially expressed genes. Nodes identified as non-hub-bottlenecks and hub-bottlenecks (based on *degree* and *betweenness* centralities), with values above +1SD of the mean, are represented as NH-B and HB, respectively. "ST", "Ca", and "CON" represent stem cell pluripotency, calcium signaling, and connector, respectively. NH-B and HB differentially expressed in at least two analyzed GSEs are underlined. Only one gene (*PTPN11*, NH-B) is differentially expressed (up-regulated) in the three analyzed GSEs. Table sorted by alphabetical order of gene symbol.

4. Discussion

In this piece, we have constructed an *in silico* interactome model resulting from the interplay between stem cell pluripotency and Ca^{2+} signaling pathways, in a network composed by 294 genes/proteins and 2995 interactions based on ‘‘Experiments’’ and ‘‘Databases’’ and a confidence score of at least 0.400 (**Fig. 1**). The newly-developed model was then subjected to further analysis by the GALANT software, showing a similar pattern of differences when relative expression of mestastatic melanoma from three microarray datasets was plotted over the expression of primary tumor samples in the “STEMCa” interactome (**Fig. 2**). Differential expression analysis of “STEMCa” model showed significant transcriptional differences in metastatic vs. primary tumor tissues with around 51% (151 out of 294) exhibiting significant changes in at least one of the studied datasets (**Supplementary table 2**). In particular, *CTNNB1*, *GNAQ*, *GSK3B*, *GSTP1*, *MAPK3*, *PPP1CC*, *PRKACA*, *SMAD4*, and *PTPN11* are de-regulated (corrected *p*-values <0.05) central genes from our model in these tissue samples (**Table 1** and **Fig. 3**), representing not only a putative group of biomarkers but also potential therapeutic targets for melanoma metastases. The observed transcriptional changes may account for the molecular changes involved in metastatic transformation of primary tumor sites.

Melanoma is the most lethal type of skin cancer with around 73,870 estimated new cases in 2015; 9,940 people died due to this disease representing 4.5 % of all new cancer cases and its incidence is rising [25]. Metastasis is the spread of cancer cells from their primary tumor to distant organs and accounts for more than 90% of deaths in cancer patients since the majority of metastases are undetectable by clinicians [3,26]. Therefore, better comprehension of the mechanisms governing melanoma metastasis and the discovery of optimal biomarkers for early diagnoses remain necessary.

In general a couple of hallmarks make CSCs putative candidates to be responsible for cancer metastases: Firstly, it seems that only CSCs within tumors are capable of initiating and maintaining cancer growth and second, a single CSC is able develop a metastatic lesion, in contrast to the rest of cancer cells from the heterogenous tumor [27,28]. In addition, it has been reported the expression of

pluripotency markers in CSCs, which share a number of the molecular characteristics typical of normal SCs and also depend on typically embryonic signaling pathways. These molecular pathways likely represent novel therapeutic targets that could improve the rates of survival in patients [29].

The homeostasis of Ca^{2+} and Ca^{2+}-dependent signaling are critical components of cellular fate [30]. Surprisingly, this event have not been studied in detail yet as in pluripotent SCs. With all, it is known the role of Ca^{2+} signaling in both proliferation and differentiation of SCs at the very early stages of development and consequently, the fate of their physiopathological status *in vivo* [31]. Therefore, one could speculate whether transcriptional differences in stem cell pluripotency and Ca^{2+} signaling-related genes may contribute to the metastatic transformation in melanoma malignancy. The results we here present, derived from our newly-developed *in silico* model (**Fig. 1** and **supplementary table 1**), corroborate such hypothesis in three independent datasets of microarray samples (**Fig. 2** and **supplementary table 2**). However, the absence of experiments confirming the up- or down-regulation of these genes at the protein level in additional human metastatic melanoma tissues remains a limitation that requires further study.

Considering that diseases treated in the early stage are the ones with the greatest probability of success, the discovery of new melanoma biomarkers (genomics, proteomics, metabolomics) with the aid of OMICS and systems biology tools are required, in order to assist clinicians in the diagnose, follow-up, treatment, and even prediction of melanoma outcomes [32]. The use of PPI offers the opportunity to analyze the functional relationships among biological molecules within one or numerous signaling pathways [16], Once alterations at the transcriptional level are confirmed, in a given biological process/es, researchers can elucidate centrality values as a network-based approach to objectively identify/select candidate biomarkers and therapeutic targets *in silico* in the context of any disease [16,33,34]. In our study, we identified a group of central members such as *CTNNB1*, *GNAQ*, *GSK3B*, *GSTP1*, *MAPK3*, *PPP1CC*, *PRKACA*, *SMAD4*, and *PTPN11* genes within our *in silico* "STEMCa" interatomic model (**Fig. 3**). Thereafter, we qualitatively validated in at least two

independent datasets of patients from GEO significant differences in expression (corrected *p*-value <0.05; FDR) (**Table 1**). From those, only *PTPN11* (NH-B) gene confirmed its up-regulation in the three cohorts of patients. This could be due to the lower number of samples provided in the third re-analyzed dataset with 58 samples (GSE15605), when compared with the other two GSEs with 83 and 104 samples, respectively. This group of nine genes/proteins, as central (NH-Bs and HBs) de-regulated nodes occupying "vulnerable" points of "STEMCa" interactome, could represent potential therapeutic targets for melanoma metastases in silico, and invites for further investigation *in vitro* and *in vivo*.

PTPN11 gene encodes for the protein tyrosine phosphatase non-receptor type 11 and has subnetwork contribution to the "STEMCa" interactome from the stem cell pluripotency signaling pathway (map04550) (**Supplementary table 1**). It is remarkable that suppression of PTPN11 confers sensitivity to BRAF inhibitors (used in cancer therapeutics) in colon cancer, by blocking signaling from receptor tyrosine kinases to the RAS-MEK-ERK pathway. This suppression also prevents the acquired resistance to targeted cancer drugs that results from receptor tyrosine kinase activation [35]. According to the CSC theory, one of the main characteristics of these cells is confer drug-resistant recurrence [1,2] and these recent findings [35] are therefore consistent with network-based identification of biomarkers and potential therapeutic targets from stem cell pluripotency and calcium signaling pathways in metastatic melanomas.

We conclude that (**i**) altered expression of stem cell pluripotency and Ca^{2+} signaling pathways-related genes may contribute to the metastatic transformation, and (**ii**) *CTNNB1*, *GNAQ*, *GSK3B*, *GSTP1*, *MAPK3*, *PPP1CC*, *PRKACA*, *SMAD4*, and *especially PTPN11* may represent an optimal candidate group of biomarkers and in *silico* therapeutic targets for melanoma metastasis.

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6. Supplementary Material

Supplementary table 1. Genes/proteins belonging to the stem cell pluripotency (map04550) and calcium signaling (map04020) interaction network model ("STEMCa" network) and its gene subnetwork contributions (stem cell pluripotency, "ST"; calcium signaling "Ca"; connector "CON").

Gene Symbol	Ensemble ID	Description	Subnetwork Contribution
ACTB	ENSP00000349960	Actin, beta	CON
ACVR1	ENSP00000263640	Activin A receptor, type I	ST
ACVR2A	ENSP00000241416	Activin A receptor, type IIA	ST
ACVR2B	ENSP00000340361	Activin A receptor, type IIB	ST
ADCY1	ENSP00000297323	Adenylate cyclase 1 (brain)	Ca
ADCY2	ENSP00000342952	Adenylate cyclase 2 (brain)	Ca
ADCY3	ENSP00000260600	Adenylate cyclase 3	Ca
ADCY4	ENSP00000312126	Adenylate cyclase 4	Ca
ADCY7	ENSP00000254235	Adenylate cyclase 7	Ca
ADCY8	ENSP00000286355	Adenylate cyclase 8 (brain)	Ca
ADCY9	ENSP00000294016	Adenylate cyclase 9	Ca
ADORA2A	ENSP00000336630	Adenosine A2a receptor	Ca
ADORA2B	ENSP00000304501	Adenosine A2b receptor	Ca
ADRA1A	ENSP00000369960	Adrenoceptor alpha 1A	Ca
ADRA1B	ENSP00000306662	Adrenoceptor alpha 1B	Ca
ADRA1D	ENSP00000368766	Adrenoceptor alpha 1D	Ca
ADRB1	ENSP00000358301	Adrenoceptor beta 1	Ca
ADRB2	ENSP00000305372	Adrenoceptor beta 2	Ca
ADRB3	ENSP00000343782	Adrenoceptor beta 3	Ca
AGTR1	ENSP00000273430	Angiotensin II receptor, type 1	Ca
AKAP10	ENSP00000225737	A kinase (PRKA) anchor protein 10	Ca
AKT1	ENSP00000270202	v-akt murine thymoma viral oncogene homolog 1	ST
AKT2	ENSP00000375892	v-akt murine thymoma viral oncogene homolog 2	ST
AKT3	ENSP00000263826	v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma)	ST
AKTIP	ENSP00000378152	AKT interacting protein	ST
ALCAM	ENSP00000305988	Activated leukocyte cell adhesion molecule	ST
ALDH1B1	ENSP00000366927	Aldehyde dehydrogenase 1 family, member B1	ST
ALDH3A1	ENSP00000225740	Aldehyde dehydrogenase 3 family, member A1	ST
APC	ENSP00000257430	Adenomatous polyposis coli	ST
ARAF	ENSP00000366244	v-raf murine sarcoma 3611 viral	ST

		oncogene homolog	
ATP2A1	ENSP00000349595	ATPase, Ca++ transporting, cardiac muscle, fast twitch 1	Ca
ATP2A2	ENSP00000440045	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	Ca
AVP	ENSP00000369647	Arginine vasopressin	CON
AVPR1A	ENSP00000299178	Arginine vasopressin receptor 1A	Ca
AVPR1B	ENSP00000356094	Arginine vasopressin receptor 1B	Ca
AXIN1	ENSP00000262320	Axin 1	ST
AXIN2	ENSP00000302625	Axin 2	ST
BDKRB1	ENSP00000216629	Bradykinin receptor B1	Ca
BDKRB2	ENSP00000307713	Bradykinin receptor B2	Ca
BMI1	ENSP00000365851	BMI1 polycomb ring finger oncogene	ST
BMP2	ENSP00000368104	Bone morphogenetic protein 2	ST
BMP4	ENSP00000245451	Bone morphogenetic protein 4	ST
BMP6	ENSP00000283147	Bone morphogenetic protein 6	ST
BMP7	ENSP00000379204	Bone morphogenetic protein 7	ST
BMPR1A	ENSP00000224764	Bone morphogenetic protein receptor, type IA	ST
BMPR1B	ENSP00000264568	Bone morphogenetic protein receptor, type IB	ST
BMPR2	ENSP00000363708	Bone morphogenetic protein receptor, type II (serine/threonine kinase)	ST
BRAF	ENSP00000288602	v-raf murine sarcoma viral oncogene homolog B1	ST
CACNA1A	ENSP00000353362	Calcium channel, voltage-dependent, P/Q type, alpha 1A subunit	Ca
CACNA1B	ENSP00000360406	Calcium channel, voltage-dependent, N type, alpha 1B subunit	Ca
CACNA1C	ENSP00000266376	Calcium channel, voltage-dependent, L type, alpha 1C subunit	Ca
CACNA1D	ENSP00000288139	Calcium channel, voltage-dependent, L type, alpha 1D subunit	Ca
CACNA1E	ENSP00000356545	Calcium channel, voltage-dependent, R type, alpha 1E subunit	Ca
CACNA1F	ENSP00000365441	Calcium channel, voltage-dependent, L type, alpha 1F subunit	Ca
CACNA1G	ENSP00000352011	Calcium channel, voltage-dependent, T type, alpha 1G subunit	Ca
CACNA1H	ENSP00000334198	Calcium channel, voltage-dependent, T type, alpha 1H subunit	Ca
CACNA1I	ENSP00000385019	Calcium channel, voltage-dependent, T type, alpha 1I subunit	Ca
CACNA1S	ENSP00000355192	Calcium channel, voltage-dependent, L type, alpha 1S subunit	Ca
CALM1	ENSP00000349467	Calmodulin 1 (phosphorylase kinase, delta)	CON
CALM2	ENSP00000272298	Calmodulin 2 (phosphorylase kinase, delta)	Ca

CAMK2B	ENSP00000379098	Calcium/calmodulin-dependent protein kinase II beta	Ca
CAMK4	ENSP00000282356	Calcium/calmodulin-dependent protein kinase IV	Ca
CCKAR	ENSP00000295589	Cholecystokinin A receptor	Ca
CCKBR	ENSP00000335544	Cholecystokinin B receptor	Ca
CCND1	ENSP00000227507	Cyclin D1	CON
CD38	ENSP00000226279	CD38 molecule	Ca
CD44	ENSP00000398632	CD44 molecule (Indian blood group)	ST
CD6	ENSP00000323280	CD6 molecule	ST
CDH5	ENSP00000344115	Cadherin 5, type 2 (vascular endothelium)	CON
CHRM1	ENSP00000306490	Cholinergic receptor, muscarinic 1	Ca
CHRM2	ENSP00000319984	Cholinergic receptor, muscarinic 2	Ca
CHRM3	ENSP00000255380	Cholinergic receptor, muscarinic 3	Ca
CHRM5	ENSP00000372750	Cholinergic receptor, muscarinic 5	Ca
CHRNA7	ENSP00000407546	Cholinergic receptor, nicotinic, alpha 7 (neuronal)	Ca
CTNNA1	ENSP00000304669	Catenin (cadherin-associated protein), alpha 1, 102kDa	ST
CTNNA2	ENSP00000418191	Catenin (cadherin-associated protein), alpha 2	ST
CTNNA3	ENSP00000362849	Catenin (cadherin-associated protein), alpha 3	ST
CTNNB1	ENSP00000344456	Catenin (cadherin-associated protein), beta 1, 88kDa	ST
CTNNBIP1	ENSP00000366466	Catenin, beta interacting protein 1	ST
CTNND2	ENSP00000307134	Catenin (cadherin-associated protein), delta 2	ST
CYSLTR1	ENSP00000362401	Cysteinyl leukotriene receptor 1	Ca
CYSLTR2	ENSP00000282018	Cysteinyl leukotriene receptor 2	Ca
DLX5	ENSP00000222598	Distal-less homeobox 5	ST
DRD1	ENSP00000327652	Dopamine receptor D1	Ca
DRD5	ENSP00000306129	Dopamine receptor D5	Ca
DVL1	ENSP00000368169	Dishevelled, dsh homolog 1 (Drosophila)	ST
EDNRA	ENSP00000315011	Endothelin receptor type A	Ca
EDNRB	ENSP00000366416	Endothelin receptor type B	Ca
EGFR	ENSP00000275493	Epidermal growth factor receptor	Ca
ERBB2	ENSP00000269571	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	Ca
ERBB3	ENSP00000267101	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	Ca
ERBB4	ENSP00000342235	v-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian)	Ca
ESR1	ENSP00000206249	Estrogen receptor 1	CON
ETV4	ENSP00000321835	Ets variant 4	ST

ETV5	ENSP00000306894	Ets variant 5	ST
F2R	ENSP00000321326	Coagulation factor II (thrombin) receptor	Ca
FGF2	ENSP00000264498	Fibroblast growth factor 2 (basic)	ST
FGFR1	ENSP00000393312	Fibroblast growth factor receptor 1	ST
FGFR2	ENSP00000410294	Fibroblast growth factor receptor 2	ST
FGFR3	ENSP00000339824	Fibroblast growth factor receptor 3	ST
FGFR4	ENSP00000292408	Fibroblast growth factor receptor 4	ST
FZD1	ENSP00000287934	Frizzled family receptor 1	ST
FZD10	ENSP00000229030	Frizzled family receptor 10	ST
FZD2	ENSP00000323901	Frizzled family receptor 2	ST
FZD3	ENSP00000240093	Frizzled family receptor 3	ST
FZD4	ENSP00000434034	Frizzled family receptor 4	ST
FZD5	ENSP00000354607	Frizzled family receptor 5	ST
FZD6	ENSP00000351605	Frizzled family receptor 6	ST
FZD7	ENSP00000286201	Frizzled family receptor 7	ST
FZD8	ENSP00000363826	Frizzled family receptor 8	ST
FZD9	ENSP00000345785	Frizzled family receptor 9	ST
GABRR1	ENSP00000412673	Gamma-aminobutyric acid (GABA) A receptor, rho 1	CON
GDF5	ENSP00000363489	Growth differentiation factor 5	ST
GNA11	ENSP00000078429	Guanine nucleotide binding protein (G protein), alpha 11 (Gq class)	Ca
GNA14	ENSP00000365807	Guanine nucleotide binding protein (G protein), alpha 14	Ca
GNA15	ENSP00000262958	Guanine nucleotide binding protein (G protein), alpha 15 (Gq class)	Ca
GNAL	ENSP00000334051	Guanine nucleotide binding protein (G protein), alpha activating activity polypeptide, olfactory type	Ca
GNAQ	ENSP00000286548	Guanine nucleotide binding protein (G protein), q polypeptide	Ca
GNAS	ENSP00000360141	GNAS complex locus	Ca
GRB2	ENSP00000339007	Growth factor receptor-bound protein 2	ST
GRIN1	ENSP00000360608	Glutamate receptor, ionotropic, N-methyl D-aspartate 1	Ca
GRIN2A	ENSP00000332549	Glutamate receptor, ionotropic, N-methyl D-aspartate 2A	Ca
GRIN2C	ENSP00000293190	Glutamate receptor, ionotropic, N-methyl D-aspartate 2C	Ca
GRIN2D	ENSP00000263269	Glutamate receptor, ionotropic, N-methyl D-aspartate 2D	Ca
GRM1	ENSP00000282753	Glutamate receptor, metabotropic 1	Ca
GRM5	ENSP00000306138	Glutamate receptor, metabotropic 5	Ca
GRPR	ENSP00000369643	Gastrin-releasing peptide receptor	Ca
GSK3B	ENSP00000324806	Glycogen synthase kinase 3 beta	ST
GSTP1	ENSP00000381607	Glutathione S-transferase pi 1	CON
HAND1	ENSP00000231121	Heart and neural crest derivatives expressed 1	ST

HDAC1	ENSP00000362649	Histone deacetylase 1	CON
HESX1	ENSP00000295934	HESX homeobox 1	ST
HOXB1	ENSP00000355140	Homeobox B1	ST
HRAS	ENSP00000309845	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	ST
HRH1	ENSP00000380247	Histamine receptor H1	Ca
HRH2	ENSP00000366506	Histamine receptor H2	Ca
HTR2A	ENSP00000367959	5-hydroxytryptamine (serotonin) receptor 2A, G protein-coupled	Ca
HTR4	ENSP00000353915	5-hydroxytryptamine (serotonin) receptor 4, G protein-coupled	Ca
HTR6	ENSP00000289753	5-hydroxytryptamine (serotonin) receptor 6, G protein-coupled	Ca
HTR7	ENSP00000337949	5-hydroxytryptamine (serotonin) receptor 7, adenylate cyclase-coupled	Ca
ID1	ENSP00000365280	Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	ST
ID2	ENSP00000234091	Inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	ST
ID3	ENSP00000363689	Inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	ST
ID4	ENSP00000367972	Inhibitor of DNA binding 4, dominant negative helix-loop-helix protein	ST
IGF1	ENSP00000302665	Insulin-like growth factor 1 (somatomedin C)	CON
IGFBP1	ENSP00000275525	Insulin-like growth factor binding protein 1	ST
IGFBP2	ENSP00000233809	Insulin-like growth factor binding protein 2, 36kDa	ST
IGFBP3	ENSP00000370473	Insulin-like growth factor binding protein 3	ST
IGFBP4	ENSP00000269593	Insulin-like growth factor binding protein 4	ST
IGFBP5	ENSP00000233813	Insulin-like growth factor binding protein 5	ST
IGFBP6	ENSP00000301464	Insulin-like growth factor binding protein 6	ST
IGFBP7	ENSP00000295666	Insulin-like growth factor binding protein 7	ST
ISL1	ENSP00000230658	ISL LIM homeobox 1	ST
ITPR1	ENSP00000306253	Inositol 1,4,5-trisphosphate receptor, type 1	Ca
ITPR2	ENSP00000370744	Inositol 1,4,5-trisphosphate receptor, type 2	Ca
ITPR3	ENSP00000363435	Inositol 1,4,5-trisphosphate receptor, type 3	Ca
JARID2	ENSP00000341280	Jumonji, AT rich interactive domain 2	ST
JUN	ENSP00000360266	Jun proto-oncogene	CON
KAT2B	ENSP00000263754	K(lysine) acetyltransferase 2B	CON

KAT6A	ENSP00000265713	K(lysine) acetyltransferase 6A	ST
KIT	ENSP00000288135	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	ST
KLF4	ENSP00000363804	Kruppel-like factor 4 (gut)	ST
LEF1	ENSP00000265165	Lymphoid enhancer-binding factor 1	ST
LEFTY2	ENSP00000355785	Left-right determination factor 2	ST
LHCGR	ENSP00000294954	Luteinizing hormone/choriogonadotropin receptor	Ca
LIF	ENSP00000249075	Leukemia inhibitory factor	ST
LIFR	ENSP00000263409	Leukemia inhibitory factor receptor alpha	ST
LTB4R2	ENSP00000433290	Leukotriene B4 receptor 2	Ca
LYN	ENSP00000428924	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	ST
MAOA	ENSP00000340684	Monoamine oxidase A	CON
MAP3K1	ENSP00000382423	Mitogen-activated protein kinase kinase kinase 1, E3 ubiquitin protein ligase	ST
MAPK1	ENSP00000215832	Mitogen-activated protein kinase 1	ST
MAPK11	ENSP00000333685	Mitogen-activated protein kinase 11	ST
MAPK12	ENSP00000215659	Mitogen-activated protein kinase 12	ST
MAPK13	ENSP00000211287	Mitogen-activated protein kinase 13	ST
MAPK14	ENSP00000229794	Mitogen-activated protein kinase 14	ST
MAPK3	ENSP00000263025	Mitogen-activated protein kinase 3	ST
MEIS1	ENSP00000272369	Meis homeobox 1	ST
MSC	ENSP00000321445	Musculin	ST
MYC	ENSP00000367207	v-myc myelocytomatosis viral oncogene homolog (avian)	ST
MYLK	ENSP00000353452	Myosin light chain kinase	Ca
NANOG	ENSP00000229307	Nanog homeobox	ST
NANOGP1	ENSP00000432545	Nanog homeobox pseudogene 1	ST
NCOR1	ENSP00000268712	Nuclear receptor corepressor 1	CON
NEUROG1	ENSP00000317580	Neurogenin 1	ST
NHP2	ENSP00000274606	NHP2 ribonucleoprotein homolog (yeast)	CON
NODAL	ENSP00000287139	Nodal homolog (mouse)	ST
NOS1	ENSP00000337459	Nitric oxide synthase 1 (neuronal)	Ca
NOS2	ENSP00000327251	Nitric oxide synthase 2, inducible	Ca
NOS3	ENSP00000297494	Nitric oxide synthase 3 (endothelial cell)	Ca
NRAS	ENSP00000358548	Neuroblastoma RAS viral (v-ras) oncogene homolog	ST
NTSR1	ENSP00000359532	Neurotensin receptor 1 (high affinity)	Ca
ONECUT1	ENSP00000302630	One cut homeobox 1	ST
ORAI1	ENSP00000328216	ORAI calcium release-activated calcium modulator 1	Ca
ORAI2	ENSP00000348752	ORAI calcium release-activated calcium modulator 2	Ca
ORAI3	ENSP00000322249	ORAI calcium release-activated calcium modulator 3	Ca
OTX1	ENSP00000282549	Orthodenticle homeobox 1	ST

OXTR	ENSP00000324270	Oxytocin receptor	Ca
P2RX1	ENSP00000225538	Purinergic receptor P2X, ligand-gated ion channel, 1	Ca
P2RX2	ENSP00000343339	Purinergic receptor P2X, ligand-gated ion channel, 2	Ca
P2RX3	ENSP00000263314	Purinergic receptor P2X, ligand-gated ion channel, 3	Ca
P2RX4	ENSP00000336607	Purinergic receptor P2X, ligand-gated ion channel, 4	Ca
P2RX6	ENSP00000416193	Purinergic receptor P2X, ligand-gated ion channel, 6	Ca
P2RX7	ENSP00000442349	Purinergic receptor P2X, ligand-gated ion channel, 7	Ca
PAX6	ENSP00000368401	Paired box 6	ST
PDGFRA	ENSP00000257290	Platelet-derived growth factor receptor, alpha polypeptide	Ca
PDGFRB	ENSP00000261799	Platelet-derived growth factor receptor, beta polypeptide	Ca
PHKG1	ENSP00000297373	Phosphorylase kinase, gamma 1 (muscle)	Ca
PIK3CG	ENSP00000352121	Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit gamma	ST
PIK3R1	ENSP00000274335	Phosphoinositide-3-kinase, regulatory subunit 1 (alpha)	ST
PIK3R5	ENSP00000392812	Phosphoinositide-3-kinase, regulatory subunit 5	ST
PLCB1	ENSP00000338185	Phospholipase C, beta 1 (phosphoinositide-specific)	Ca
PLCB2	ENSP00000260402	Phospholipase C, beta 2	Ca
PLCB3	ENSP00000279230	Phospholipase C, beta 3 (phosphatidylinositol-specific)	Ca
PLCB4	ENSP00000334105	Phospholipase C, beta 4	Ca
PLCD1	ENSP00000430344	Phospholipase C, delta 1	Ca
PLCD3	ENSP00000313731	Phospholipase C, delta 3	Ca
PLCD4	ENSP00000388631	Phospholipase C, delta 4	Ca
PLCE1	ENSP00000260766	Phospholipase C, epsilon 1	Ca
PLCG1	ENSP00000244007	Phospholipase C, gamma 1	Ca
PLN	ENSP00000350132	Phospholamban	Ca
POU5F1	ENSP00000259915	POU class 5 homeobox 1	ST
PPIF	ENSP00000225174	Peptidylprolyl isomerase F	Ca
PPP1CC	ENSP00000335084	Protein phosphatase 1, catalytic subunit, gamma isozyme	CON
PRC1	ENSP00000377793	Protein regulator of cytokinesis 1	ST
PRKACA	ENSP00000309591	Protein kinase, cAMP-dependent, catalytic, alpha	CON
PRKCA	ENSP00000408695	Protein kinase C, alpha	Ca
PRKCB	ENSP00000305355	Protein kinase C, beta	Ca
PRKCG	ENSP00000263431	Protein kinase C, gamma	Ca
PROM1	ENSP00000415481	Prominin 1	ST

PTAFR	ENSP00000301974	Platelet-activating factor receptor	Ca
PTEN	ENSP00000361021	Phosphatase and tensin homolog	ST
PTGER1	ENSP00000292513	Prostaglandin E receptor 1 (subtype EP1), 42kDa	Ca
PTGER3	ENSP00000349003	Prostaglandin E receptor 3 (subtype EP3)	Ca
PTGFR	ENSP00000359793	Prostaglandin F receptor (FP)	Ca
PTK2B	ENSP00000332816	PTK2B protein tyrosine kinase 2 beta	Ca
PTPN11	ENSP00000340944	Protein tyrosine phosphatase, non-receptor type 11	ST
RAF1	ENSP00000251849	v-raf-1 murine leukemia viral oncogene homolog 1	ST
REST	ENSP00000311816	RE1-silencing transcription factor	ST
RIF1	ENSP00000243326	RAP1 interacting factor homolog (yeast)	ST
RRAS	ENSP00000246792	Related RAS viral (r-ras) oncogene homolog	ST
RYR1	ENSP00000352608	Ryanodine receptor 1 (skeletal)	Ca
RYR2	ENSP00000355533	Ryanodine receptor 2 (cardiac)	Ca
RYR3	ENSP00000373884	Ryanodine receptor 3	Ca
SALL4	ENSP00000217086	Sal-like 4 (Drosophila)	CON
SDC1	ENSP00000254351	Syndecan 1	CON
SETDB1	ENSP00000271640	SET domain, bifurcated 1	ST
SKIL	ENSP00000259119	SKI-like oncogene	ST
SLC25A4	ENSP00000281456	Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4	Ca
SMAD2	ENSP00000262160	SMAD family member 2	ST
SMAD3	ENSP00000332973	SMAD family member 3	ST
SMAD4	ENSP00000341551	SMAD family member 4	ST
SMAD6	ENSP00000288840	SMAD family member 6	ST
SMAD7	ENSP00000262158	SMAD family member 7	ST
SMARCAD1	ENSP00000351947	SWI/SNF-related, matrix-associated actin-dependent regulator of chromatin, subfamily a, containing DEAD/H box 1	ST
SOX10	ENSP00000354130	SRY (sex determining region Y)-box 10	CON
SOX2	ENSP00000323588	SRY (sex determining region Y)-box 2	ST
SPHK1	ENSP00000313681	Sphingosine kinase 1	Ca
STAT3	ENSP00000264657	Signal transducer and activator of transcription 3 (acute-phase response factor)	ST
STIM1	ENSP00000300737	Stromal interaction molecule 1	Ca
STIM2	ENSP00000417569	Stromal interaction molecule 2	Ca
TACR1	ENSP00000303522	Tachykinin receptor 1	Ca
TACR2	ENSP00000362403	Tachykinin receptor 2	Ca
TACR3	ENSP00000303325	Tachykinin receptor 3	Ca
TBX3	ENSP00000257566	T-box 3	ST
TBXA2R	ENSP00000393333	Thromboxane A2 receptor	Ca
TCF3	ENSP00000262965	Transcription factor 3 (E2A immunoglobulin enhancer binding	ST

		factors E12/E47)	
THY1	ENSP00000284240	Thy-1 cell surface antigen	ST
TLE4	ENSP00000365735	Transducin-like enhancer of split 4 (E(sp1) homolog, Drosophila)	CON
TRHR	ENSP00000309818	Thyrotropin-releasing hormone receptor	Ca
VDAC1	ENSP00000265333	Voltage-dependent anion channel 1	Ca
VDAC2	ENSP00000361635	Voltage-dependent anion channel 2	Ca
VDAC3	ENSP00000428845	Voltage-dependent anion channel 3	Ca
WNT1	ENSP00000293549	Wingless-type MMTV integration site family, member 1	ST
WNT10A	ENSP00000258411	Wingless-type MMTV integration site family, member 10A	ST
WNT10B	ENSP00000301061	Wingless-type MMTV integration site family, member 10B	ST
WNT11	ENSP00000325526	Wingless-type MMTV integration site family, member 11	ST
WNT16	ENSP00000222462	Wingless-type MMTV integration site family, member 16	ST
WNT2	ENSP00000265441	Wingless-type MMTV integration site family member 2	ST
WNT2B	ENSP00000358698	Wingless-type MMTV integration site family, member 2B	ST
WNT3	ENSP00000225512	Wingless-type MMTV integration site family, member 3	ST
WNT3A	ENSP00000284523	Wingless-type MMTV integration site family, member 3A	ST
WNT4	ENSP00000290167	Wingless-type MMTV integration site family, member 4	ST
WNT5A	ENSP00000264634	Wingless-type MMTV integration site family, member 5A	ST
WNT5B	ENSP00000308887	Wingless-type MMTV integration site family, member 5B	ST
WNT6	ENSP00000233948	Wingless-type MMTV integration site family, member 6	ST
WNT7A	ENSP00000285018	Wingless-type MMTV integration site family, member 7A	ST
WNT7B	ENSP00000341032	Wingless-type MMTV integration site family, member 7B	ST
WNT8A	ENSP00000381739	Wingless-type MMTV integration site family, member 8A	ST
WNT8B	ENSP00000340677	Wingless-type MMTV integration site family, member 8B	ST
WNT9A	ENSP00000272164	Wingless-type MMTV integration site family, member 9A	ST
WNT9B	ENSP00000290015	Wingless-type MMTV integration site family, member 9B	ST
ZIC3	ENSP00000287538	Zic family member 3	ST
ZNF593	ENSP00000363384	Zinc finger protein 593	ST

Supplementary table 2. Centrality values (*degree* and *betweenness*) and differentially expressed genes from the stem cell pluripotency (map04550) and calcium signaling (map04020) interaction network model ("STEMCa" network). Corrected *p*-values <0.05 were considered significant. "ST", "Ca", and "CON" represent stem cell pluripotency, calcium signaling, and connector, respectively. Centralities over the thresholds with value/s above one (+1SD) standard deviation of the mean are color-marked.

GENE INFORMATION			**TOPOLOGY**		**GSE8401**			**GSE46517**			**GSE15605**		
Gene Symbol	**Ensemble ID**	**Subnetwork**	***Degree***	***Betweenness***	**LogFC**	**Diff. Exp.**	**Corrected *p*-value** (FDR)	**LogFC**	**Diff Exp**	**Corrected *p*-value** (FDR)	**LogFC**	**Diff. Exp.**	**Corrected *p*-value** (FDR)
ACTB	ENSP00000349960	CON	20	0.01907129	-0.024108298	no	0.761449925	-0.00686	no	0.803684421	1.559675026	**Up**	**0.032652195**
ACVR1	ENSP00000263640	ST	11	0.00718543	0.493435774	**Up**	**0.00234 1069**	0.014872	no	0.750579897	0.259755304	no	0.395003714
ACVR2A	ENSP00000241416	ST	15	0.00119467	-0.028341 3	no	0.677864084	0.017547	no	0.634267226	-0.456205804	no	0.088499856
ACVR2B	ENSP00000340361	ST	15	0.00119467	0.125032579	no	0.105642614	0.024948	no	0.467903 1	0.475372468	no	0.181619371
ADCY1	ENSP00000297323	Ca	28	0.00226065	0.770309904	**Up**	**0.00323 7809**	0.129314	**Up**	**0.04036285**	0.250179176	no	0.8225865
ADCY2	ENSP00000342952	Ca	31	0.00377255	-0.816673844	**Down**	**0.00091 3162**	-0.3964	**Down**	**4.27246 E-06**	-2.246747858	**Down**	**0.019316464**
ADCY3	ENSP00000260600	Ca	32	0.00459359	0.063873407	no	0.637425296	-0.041 02	no	0.23974253	-0.130007519	no	0.769024733
ADCY4	ENSP00000312126	Ca	31	0.00377255	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	-0.963961392	**Down**	**0.047564684**
ADCY7	ENSP00000254235	Ca	32	0.01277469	-0.047589167	no	0.816569602	-0.07034	no	0.408955104	-0.210228945	no	0.601933035
ADCY8	ENSP00000286355	Ca	33	0.00446963	-0.174215996	**Down**	**0.01661 6289**	-0.06279	no	0.051168453	-0.335921631	no	0.247837447
ADCY9	ENSP00000294016	Ca	31	0.00377255	-0.428191131	**Down**	**0.01814 938**	-0.18131	**Down**	**0.01507 8196**	-0.519722729	no	0.103479907
ADORA2A	ENSP00000336630	Ca	23	0.00102844	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*
ADORA2B	ENSP00000304501	Ca	22	0.000576	0.091943694	no	0.677859748	-0.03734	no	0.526103972	0.552891787	no	0.443724055
ADRA1A	ENSP00000369960	Ca	**43**	0.000634	-0.052828498	no	0.319141538	-0.07484	no	0.21525575	-0.206687591	no	0.347422003
ADRA1B	ENSP00000306662	Ca	**46**	0.00127274	-0.056029792	no	0.299706925	0.029342	no	0.650094 66	-0.111464079	no	0.586795269
ADRA1D	ENSP00000368766	Ca	**43**	0.000634	-0.073784386	no	0.379436234	-0.0444	no	0.377800315	-0.204841053	no	0.424114462
ADRB1	ENSP00000358301	Ca	24	0.00197701	-0.110653483	no	0.054753207	0.024246	no	0.629602 4	0.675856475	no	0.416422134
ADRB	ENSP00000	Ca	29	0.00258	-	no	0.06054	-	no	0.18549	-	**Do**	**0.0131**

2	305372			616	0.23687 8921		8662	0.041 27		4082	1.52973 6243	**wn**	**42131**
ADRB 3	ENSP00000 343782	Ca	21	0.00000 275	- 0.02450 0254	no	0.74364 1898	- 0.100 18	no	0.05300 0578	- 0.38532 8567	no	0.1007 19202
AGTR 1	ENSP00000 273430	Ca	**48**	0.00448 218	- 0.05267 8124	no	0.73132 5344	0.088 783	no	0.22338 8799	0.24448 0193	no	0.7743 08308
AKAP 10	ENSP00000 225737	Ca	1	0	0.11176 6375	no	0.14278 6753	0.084 407	no	0.05202 8474	0.87140 7694	no	0.1485 02956
AKT1	ENSP00000 270202	ST	32	**0.03034 247**	- 0.24592 9347	no	0.07309 4859	- 0.118 25	**Do wn**	**0.00257 2769**	- 0.00455 5422	no	0.9915 9187
AKT2	ENSP00000 375892	ST	10	0.00172 914	0.00368 7556	no	0.97038 7807	- 0.054 44	no	0.41407 7105	0.47289 4843	no	0.2143 53755
AKT3	ENSP00000 263826	ST	6	0.00009 74	0.53815 105	**Up**	**0.00024 0754**	0.019 771	no	0.80083 1597	1.94695 9962	**Up**	**0.0042 71082**
AKTIP	ENSP00000 378152	ST	1	0	- 0.19260 4401	**Do wn**	**0.01898 5222**	- 0.060 75	no	0.07928 4466	0.14151 5237	no	0.6875 92363
ALCA M	ENSP00000 305988	ST	1	0	0.01430 6297	no	0.96087 0922	- 0.072 82	no	0.52421 4628	- 0.03479 6199	no	0.9618 46632
ALDH 1B1	ENSP00000 366927	ST	1	0	0.27180 7519	**Up**	**0.01847 9944**	0.084 034	no	0.10839 2189	0.17344 8261	no	0.7463 13451
ALDH 3A1	ENSP00000 225740	ST	2	0.01360 512	- 1.28140 477	**Do wn**	**7.65086 E-14**	- 0.369 27	**Do wn**	**1.00595 E-14**	- 1.66618 2948	**Do wn**	**0.0124 65938**
APC	ENSP00000 257430	ST	13	0.00330 135	0.04445 2604	no	0.39078 6804	0.071 77	no	0.17291 3743	0.84023 5375	no	0.1442 16088
ARAF	ENSP00000 366244	ST	10	0.00009 34	0.10216 7477	no	0.13460 5848	- 0.020 64	no	0.57008 4586	0.50696 9343	no	0.1907 50032
ATP2A 1	ENSP00000 349595	Ca	2	0.00005 75	- 0.03143 3038	no	0.74094 2807	- 0.023 71	no	0.74313 0158	- 0.25413 4846	no	0.2180 62139
ATP2A 2	ENSP00000 440045	Ca	3	0.00022 2	0.22505 9947	no	0.07450 3034	0.003 918	no	0.94478 3973	0.22349 7694	no	0.4151 18732
AVP	ENSP00000 369647	CON	**63**	0.01721 403	- 0.16039 8739	**Do wn**	**0.00835 7143**	0.039 159	no	0.36406 6614	- 0.08256 2994	no	0.6130 46328
AVPR 1A	ENSP00000 299178	Ca	**43**	0.00037 3	0.02537 9757	no	0.72690 2328	- 0.072 53	no	0.21341 6041	- 0.16885 8597	no	0.7427 0437
AVPR 1B	ENSP00000 356094	Ca	1	0	- 0.19423 6639	**Do wn**	**0.00206 9273**	0.017 751	no	0.63398 8754	- 0.15209 6423	no	0.4414 88605
AXIN1	ENSP00000 262320	ST	21	0.01043 37	- 0.10224 1516	no	0.08114 5394	- 0.039 99	no	0.13260 8851	- 0.34316 5433	no	0.2251 50251
AXIN2	ENSP00000 302625	ST	9	0.00023 8	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	- 1.33914 3633	**Do wn**	**0.0154 7958**
BDKR B1	ENSP00000 216629	Ca	**51**	0.00208 106	- 0.14115 1364	**Do wn**	**0.01723 9378**	- 0.053 64	no	0.49368 9279	- 0.36495 9628	no	0.3227 24166
BDKR B2	ENSP00000 307713	Ca	**54**	0.00547 469	- 0.67796 8642	**Do wn**	**1.24357 E-15**	- 0.420 07	**Do wn**	**1.38507 E-17**	- 1.05612 7029	**Do wn**	**0.0451 21581**
BMI1	ENSP00000 365851	ST	4	0.00003 58	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*
BMP2	ENSP00000 368104	ST	20	0.00935 5	- 0.22259 0883	no	0.29776 4194	0.043 115	no	0.59259 277	- 1.28833 4655	**Do wn**	**0.0158 89038**
BMP4	ENSP00000 245451	ST	16	0.00731 462	- 0.31036 2702	**Do wn**	**0.00309 9308**	- 0.471 96	**Do wn**	**4.18036 E-05**	- 0.81379 411	no	0.0949 84367
BMP6	ENSP00000 283147	ST	7	0.00000 425	0.12308 4462	no	0.28764 1243	0.093 497	no	0.16719 5897	0.52850 6397	no	0.2642 63554
BMP7	ENSP00000	ST	11	0.00017	-	no	0.24368	-	no	0.46218	0.07807	no	0.9557

	379204			4	0.083799725		6624	0.03942		0145	5942		06107
BMPR1A	ENSP00000224764	ST	14	0.000735	0.145059004	no	0.059506879	-0.01856	no	0.827340048	0.574343394	no	0.120578819
BMPR1B	ENSP00000264568	ST	13	0.000659	0.130798092	no	0.136287556	0.069788	no	0.121814471	-1.474246454	no	0.053777794
BMPR2	ENSP00000363708	ST	14	0.000735	-0.448336832	**Down**	**0.000118576**	-0.23117	**Down**	**3.66751E-07**	0.861342009	no	0.087437272
BRAF	ENSP00000288602	ST	14	0.000595	0.186639374	**Up**	**0.00209567**	0.097903	**Up**	**0.004428605**	0.653803191	no	0.213700785
CACNA1A	ENSP00000353362	Ca	5	0.000403	-0.031591228	no	0.751838231	-0.17586	**Down**	**0.004772247**	-0.064931331	no	0.905752777
CACNA1B	ENSP00000360406	Ca	7	0	-0.24689251	**Down**	**0.001150002**	0.03364	no	0.466035081	-0.564549564	no	0.101426239
CACNA1C	ENSP00000266376	Ca	17	0.005718	-0.099400691	no	0.174228705	-0.09854	no	0.060230691	-0.41947977	no	0.374748958
CACNA1D	ENSP00000288139	Ca	16	0.00632295	-0.095407579	no	0.169386086	-0.0188	no	0.798179115	-1.19305511	no	0.086683789
CACNA1E	ENSP00000356545	Ca	7	0	-0.062648998	no	0.332426359	0.009584	no	0.862237025	-0.376419091	no	0.324810026
CACNA1F	ENSP00000365441	Ca	7	0	0.110494279	no	0.181265161	-0.05571	no	0.313058214	-0.215498325	no	0.227971683
CACNA1G	ENSP00000352011	Ca	12	0.00971323	-0.079662181	no	0.196739841	-0.03226	no	0.682084341	-0.554730986	no	0.109883555
CACNA1H	ENSP00000334198	Ca	10	0.000901	-0.147646031	**Down**	**0.018103666**	-0.04337	no	0.303674783	-0.344074896	no	0.146501819
CACNA1I	ENSP00000385019	Ca	7	0.000017	-0.030942	no	0.68026309	0.015795	no	0.715554113	-0.094909931	no	0.783393576
CACNA1S	ENSP00000355192	Ca	14	0.00431991	-0.077981502	no	0.159584796	0.049286	no	0.278558668	-0.033481743	no	0.845326922
CALM1	ENSP00000349467	CON	**46**	**0.04297522**	0.05970491	no	0.420640218	0.082097	**Up**	**0.014354422**	0.898796891	no	0.102753445
CALM2	ENSP00000272298	Ca	11	0.00310394	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*
CAMK2B	ENSP00000379098	Ca	29	0.00928585	-0.017180559	no	0.696252539	-0.0208	no	0.825450834	-0.036004175	no	0.934238294
CAMK4	ENSP00000282356	Ca	7	0.000468	0.082553391	no	0.235787616	0.150469	**Up**	**0.000955151**	0.212876403	no	0.800197653
CCKAR	ENSP00000295589	Ca	**42**	0.00000382	-0.114222594	no	0.106696824	0.013983	no	0.76901408	-0.046934205	no	0.900241907
CCKBR	ENSP00000335544	Ca	**43**	0.00127413	-0.150809949	**Down**	**0.025703471**	-0.02904	no	0.368975123	-0.423549775	no	0.165528817
CCND1	ENSP00000227507	CON	18	0.0073975	-0.786599389	**Down**	**0.00159198**	-0.22917	**Down**	**0.005067749**	-0.100126092	no	0.852703841
CD38	ENSP00000226279	Ca	6	0.000342	0.633163266	**Up**	**0.00152246**	0.14165	**Up**	**0.007978865**	1.375678242	**Up**	**0.005307694**
CD44	ENSP00000398632	ST	10	0.00229193	-0.443061465	**Down**	**0.022986638**	0.131686	no	0.179109005	0.490288672	no	0.479148798
CD6	ENSP00000323280	ST	2	0.00682594	-0.407181124	**Down**	**0.002998311**	0.067237	no	0.711281452	-0.415584687	no	0.496054969
CDH5	ENSP00000344115	CON	6	0.01369088	-0.229501486	no	0.139755758	-0.2715	**Down**	**0.000198259**	-0.305482103	no	0.390122841

CHRM1	ENSP00000306490	Ca	**49**	0.00816648	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	-0.848163445	no	0.08631478
CHRM2	ENSP00000319984	Ca	16	0.000846	0.102543657	no	0.368022426	0.148074	**Up**	**2.72041E-05**	-0.141731168	no	0.446372646
CHRM3	ENSP00000255380	Ca	**44**	0.000224	0.302635974	**Up**	**0.033554266**	0.048657	no	0.704420478	-0.257621355	no	0.776247495
CHRM5	ENSP00000372750	Ca	**44**	0.000224	0.086640245	no	0.35743343	0.064373	no	0.098808445	-0.211711882	no	0.325431383
CHRNA7	ENSP00000407546	Ca	1	0	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*
CTNNA1	ENSP00000304669	ST	16	0.00547324	-0.423084932	**Down**	**0.001525878**	-0.09298	no	0.141453598	1.150609889	no	0.060574303
CTNNA2	ENSP00000418191	ST	8	0.000443	-0.146311509	no	0.189635269	0.044375	no	0.452861071	-0.081112324	no	0.920987621
CTNNA3	ENSP00000362849	ST	3	0.0000359	-0.034442392	no	0.606649134	-0.04157	no	0.268857768	-0.177100562	no	0.422572677
CTNNB1	ENSP00000344456	ST	**49**	**0.09934346**	0.573777007	**Up**	**0.000112883**	0.161343	**Up**	**5.22113E-05**	0.801985615	no	0.134736908
CTNNBIP1	ENSP00000366466	ST	7	0.000349	-0.771669249	**Down**	**1.86249E-13**	-0.27636	**Down**	**5.36471E-16**	-0.798545079	**Down**	**0.031494238**
CTNND2	ENSP00000307134	ST	2	0.000215	-0.175894329	**Down**	**0.000467185**	0.03196	no	0.594139458	-0.568506595	no	0.275886549
CYSLTR1	ENSP00000362401	Ca	**42**	0.00000382	-0.066353691	no	0.059370283	0.042701	no	0.525517399	-0.359284529	no	0.425823745
CYSLTR2	ENSP00000282018	Ca	**42**	0.00000382	0.006319143	no	0.961072995	-0.01204	no	0.873988316	-0.645886751	no	0.233398296
DLX5	ENSP00000222598	ST	1	0	-0.268598333	**Down**	**3.33381E-05**	-0.01215	no	0.728025125	0.110247434	no	0.840731238
DRD1	ENSP00000327652	Ca	23	0.001007	0.013983884	no	0.787069697	0.102939	**Up**	**0.016480638**	-0.19250745	no	0.242391909
DRD5	ENSP00000306129	Ca	22	0.000576	0.055393592	no	0.592811441	0.005495	no	0.868778465	-0.01086879	no	0.946459053
DVL1	ENSP00000368169	ST	24	0.01655107	-0.251755594	**Down**	**0.02991641**	-0.04217	no	0.423828376	-0.879342464	**Down**	**0.004058239**
EDNRA	ENSP00000315011	Ca	**54**	0.00594781	-0.152646475	**Down**	**0.00389713**	-0.23916	**Down**	**0.000373873**	-0.463940959	no	0.454813356
EDNRB	ENSP00000366416	Ca	**45**	0.00161893	0.06962535	no	0.867511022	-0.03943	no	0.802086629	-0.165741295	no	0.788352776
EGFR	ENSP00000275493	Ca	**41**	**0.02867496**	-0.074739452	no	0.454469806	-0.77396	**Down**	**4.09354E-18**	-0.912000768	no	0.372953188
ERBB2	ENSP00000269571	Ca	23	0.00599284	-0.771670613	**Down**	**4.23948E-10**	-0.29477	**Down**	**1.84539E-10**	-0.579780806	no	0.171349238
ERBB3	ENSP00000267101	Ca	14	0.00208024	0.016327019	no	0.968562333	-0.04873	no	0.682084341	-0.016214834	no	0.984742649
ERBB4	ENSP00000342235	Ca	18	0.00462692	0.145840034	no	0.060516277	-0.06295	no	0.122525084	0.129515459	no	0.898711344
ESR1	ENSP00000206249	CON	37	**0.03902762**	0.065573168	no	0.343611801	-0.03276	no	0.691913409	-0.121373719	no	0.858356832
ETV4	ENSP00000	ST	1	0	0.04648	no	0.79195	0.029	no	0.79411	1.03681	**Up**	**0.0291**

	321835				2663		8206	367		606	2913		**15616**
ETV5	ENSP00000306894	ST	1	0	0.260189791	no	0.33458146	-0.14935	no	0.068682414	1.201575161	no	0.081140066
F2R	ENSP00000321326	Ca	**48**	0.01178359	0.602243494	**Up**	**0.023147566**	0.108833	no	0.176177758	-0.534595111	no	0.346116148
FGF2	ENSP00000264498	ST	14	0.00281367	0.106923208	no	0.101486883	-0.006	no	0.946133832	0.230020218	no	0.692780856
FGFR1	ENSP00000393312	ST	16	0.00132134	-0.169012033	no	0.385036119	-0.17179	no	0.059545658	0.021948761	no	0.973762337
FGFR2	ENSP00000410294	ST	16	0.00237305	-0.043788263	no	0.546662231	-0.49196	**Down**	**2.8766E-13**	-2.893741608	**Down**	**0.006270883**
FGFR3	ENSP00000339824	ST	10	0.000465	-2.51545166	**Down**	**4.2596E-13**	-1.11192	**Down**	**7.5847E-19**	-3.214335236	**Down**	**0.006124764**
FGFR4	ENSP00000292408	ST	9	0.000477	0.084377835	no	0.230262848	0.147837	**Up**	**0.026484362**	-0.273961519	no	0.390185329
FZD1	ENSP00000287934	ST	25	0.00690974	0.269871118	no	0.255895831	0.040916	no	0.694950224	0.648658056	no	0.0820422
FZD10	ENSP00000229030	ST	21	0.00437685	-0.715983427	**Down**	**2.49109E-11**	-0.32664	**Down**	**3.50974E-15**	-1.526629405	**Down**	**0.023253115**
FZD2	ENSP00000323901	ST	28	0.00702029	-0.088095883	no	0.410641519	-0.00588	no	0.920622314	0.559371415	no	0.081095083
FZD3	ENSP00000240093	ST	25	0.00437685	0.243648857	**Up**	**0.007698645**	0.132634	**Up**	**0.000386567**	0.600345554	no	0.184691037
FZD4	ENSP00000434034	ST	27	0.00438291	-0.035353176	no	0.70435913	-0.04727	no	0.075469187	-0.403310645	no	0.245860687
FZD5	ENSP00000354607	ST	31	0.0172332	-0.072368926	no	0.291494394	0.064076	no	0.14874996	0.394866852	no	0.355591884
FZD6	ENSP00000351605	ST	25	0.00437685	0.476450171	no	0.075135475	-0.01869	no	0.839819858	-0.500216734	no	0.254136444
FZD7	ENSP00000286201	ST	25	0.00437986	0.42773233	no	0.080845101	0.05132	no	0.647617036	-0.180184209	no	0.750111855
FZD8	ENSP00000363826	ST	25	0.0063776	-0.040342841	no	0.67344977	0.001689	no	0.977696363	0.184641108	no	0.814632786
FZD9	ENSP00000345785	ST	3	0	-0.065039336	no	0.582377552	0.056973	no	0.363924495	0.019023728	no	0.966747866
GABRR1	ENSP00000412673	CON	4	**0.02033756**	0.043553532	no	0.516750581	0.045095	no	0.178807962	0.970277925	**Up**	**0.030236148**
GDF5	ENSP00000363489	ST	9	0.0000148	0.008037279	no	0.923245213	0.017108	no	0.800684561	-0.100366752	no	0.590368797
GNA11	ENSP00000078429	Ca	**52**	0.00607334	-0.139746345	no	0.247003933	0.412342	**Up**	**0.000111027**	0.041139788	no	0.929693492
GNA14	ENSP00000365807	Ca	**52**	0.00607334	0.087397394	no	0.235043887	-0.05462	no	0.321151731	0.175716186	no	0.746873279
GNA15	ENSP00000262958	Ca	**55**	0.0073464	-1.283552441	**Down**	**1.41761E-13**	-0.56968	**Down**	**1.24793E-15**	-1.227252208	**Down**	**0.042581416**
GNAL	ENSP00000334051	Ca	28	0.00363512	-0.611900981	**Down**	**0.003427336**	-0.69432	**Down**	**6.07879E-09**	-1.080828356	no	0.131120178
GNAQ	ENSP00000286548	Ca	**69**	**0.08508797**	0.298487114	**Up**	**0.014769785**	0.073427	**Up**	**0.038122837**	0.74299322	no	0.174777485
GNAS	ENSP00000360141	Ca	**40**	0.01161783	0.116809053	no	0.297398232	-0.08881	no	0.154063105	0.496928684	no	0.351157727
GRB2	ENSP00000339007	ST	34	0.01239059	0.1341608	no	0.173913017	0.034527	no	0.407235369	0.681520821	no	0.136051561
GRIN1	ENSP00000	Ca	18	0.00267	-	no	0.78425	-	no	0.40153	-	no	0.3624

	360608			627	0.02448 3637		3651	0.073 25		4161	0.20039 5883		22113
GRIN2 A	ENSP00000 332549	Ca	12	0.00018 5	- 0.01997 8253	no	0.82624 1727	0.023 598	no	0.65844 9765	- 1.09885 6784	**Do wn**	**0.0402 89652**
GRIN2 C	ENSP00000 293190	Ca	10	0.00008 28	- 0.06760 7325	no	0.27254 5164	0.000 465	no	0.99392 5086	- 0.03065 8879	no	0.8884 47139
GRIN2 D	ENSP00000 263269	Ca	10	0.00008 28	0.01764 0261	no	0.81872 1006	- 0.068 4	no	0.23157 3836	- 0.15393 082	no	0.8101 90058
GRM1	ENSP00000 282753	Ca	**46**	0.00168 435	0.02389 1092	no	0.67094 0189	- 0.231 28	**Do wn**	**0.00788 8872**	- 0.52683 163	no	0.2954 72353
GRM5	ENSP00000 306138	Ca	**48**	0.00373 03	- 0.09015 7207	no	0.10036 2644	0.117 849	**Up**	**0.03446 0137**	- 0.29059 2791	no	0.3455 08814
GRPR	ENSP00000 369643	Ca	**42**	0.00000 382	- 0.05554 4305	no	0.47408 8705	- 0.083 11	no	0.09045 6741	0.04510 1347	no	0.8359 32347
GSK3 B	ENSP00000 324806	ST	31	**0.03985 954**	- 0.56287 5324	**Do wn**	**4.43231 E-06**	- 0.182 47	**Do wn**	**2.75596 E-10**	- 0.20100 8769	no	0.5426 67881
GSTP1	ENSP00000 381607	CON	4	**0.02033 756**	- 0.81235 0991	**Do wn**	**3.14081 E-05**	- 0.236 41	**Do wn**	**2.61693 E-06**	0.54394 2919	no	0.2744 34846
HAND 1	ENSP00000 231121	ST	3	0.00007 25	0.07938 9499	no	0.34737 2449	0.042 779	no	0.17453 6039	- 0.26557 9967	no	0.2382 94632
HDAC 1	ENSP00000 362649	CON	27	**0.03262 084**	0.17335 5672	no	0.21343 4449	0.019 098	no	0.71099 9269	0.03820 3525	no	0.8651 72009
HESX 1	ENSP00000 295934	ST	1	0	0.04694 851	no	0.36173 578	0.060 553	no	0.33507 4905	0.52190 8034	no	0.2188 95253
HOXB 1	ENSP00000 355140	ST	2	0.00682 594	- 0.17990 224	**Do wn**	**0.00791 1256**	- 0.002 6	no	0.97030 8301	- 0.22483 583	no	0.2339 83031
HRAS	ENSP00000 309845	ST	37	0.01049 836	- 0.89099 2933	**Do wn**	**2.55324 E-09**	- 0.327 71	**Do wn**	**2.43558 E-10**	- 0.71109 7808	no	0.0778 84419
HRH1	ENSP00000 380247	Ca	**43**	0.00047	- 0.07555 4575	no	0.37426 9443	0.012 249	no	0.82734 0048	- 0.65735 8695	no	0.2266 40155
HRH2	ENSP00000 366506	Ca	21	0.00000 275	- 0.12790 8575	no	0.10141 0648	- 0.017 09	no	0.68300 89	- 0.18677 9109	no	0.3656 05641
HTR2 A	ENSP00000 367959	Ca	**45**	0.00426 483	- 0.05947 057	no	0.30275 8145	0.130 321	**Up**	**0.00431 4674**	0.03903 9577	no	0.9541 11957
HTR4	ENSP00000 353915	Ca	21	0.00000 275	- 0.17415 617	**Do wn**	**0.00587 3678**	- 0.079 07	no	0.08192 0506	- 0.18215 6995	no	0.3475 66203
HTR6	ENSP00000 289753	Ca	22	0.00057 6	- 0.07268 614	no	0.22694 529	0.027 427	no	0.61549 4184	- 0.11142 3257	no	0.6317 52012
HTR7	ENSP00000 337949	Ca	21	0.00000 275	- 0.12143 3044	**Do wn**	**0.02506 5647**	0.138 847	**Up**	**0.03184 0716**	- 0.49341 2135	no	0.0932 03046
ID1	ENSP00000 365280	ST	3	0.00017 1	- 1.20657 512	**Do wn**	**5.70183 E-08**	- 0.346 18	**Do wn**	**1.86906 E-12**	- 0.94674 1614	no	0.1663 37906
ID2	ENSP00000 234091	ST	4	0.00116 652	- 0.56693 9734	**Do wn**	**0.00239 8096**	- 0.215 76	**Do wn**	**0.00038 6567**	0.62544 8485	no	0.2625 00108
ID3	ENSP00000 363689	ST	2	0.00682 594	- 0.29889 7543	no	0.16355 4189	- 0.197	**Do wn**	**0.00857 6684**	0.25916 2096	no	0.6264 32588
ID4	ENSP00000 367972	ST	1	0	- 0.59155 1548	**Do wn**	**0.01420 0785**	- 0.199 02	**Do wn**	**0.01375 6553**	- 0.34963 0164	no	0.6113 94445
IGF1	ENSP00000 302665	CON	28	**0.04312 205**	- 0.05156 3378	no	0.69726 6013	- 0.047 49	no	0.43146 5367	0.28047 6932	no	0.6695 6775
IGFBP 1	ENSP00000 275525	ST	2	0	0.01483 7928	no	0.68326 0678	0.047 583	no	0.14714 9277	- 0.06023	no	0.8352 02534

											3499		
IGFBP 2	ENSP00000 233809	ST	1	0	0.03933 7636	no	0.91899 4593	0.027 73	no	0.84965 1004	0.95885 4082	no	0.2285 19244
IGFBP 3	ENSP00000 370473	ST	4	0.00040 7	0.13458 6986	no	0.68941 0653	0.033 758	no	0.74530 573	2.01258 2224	**Up**	**0.0020 01928**
IGFBP 4	ENSP00000 269593	ST	4	0.00227 551	-0.63924 9613	**Down**	**0.00077 7461**	-0.295 11	**Down**	**6.12729 E-05**	-0.01371 9181	no	0.9802 4487
IGFBP 5	ENSP00000 233813	ST	2	0	-0.40863 2308	no	0.12591 4093	-0.173 95	no	0.05430 6871	0.90715 9497	no	0.2465 07093
IGFBP 6	ENSP00000 301464	ST	1	0	-0.13512 7596	no	0.49825 1851	-0.044 72	no	0.47301 9	-0.19942 0337	no	0.7430 44626
IGFBP 7	ENSP00000 295666	ST	3	0.00036 9	-0.41036 2123	no	0.07293 0625	-0.246 46	**Down**	**0.00260 6339**	0.20235 2782	no	0.7765 8848
ISL1	ENSP00000 230658	ST	1	0	0.10279 5663	no	0.10800 9652	0.115 12	**Up**	**0.00541 5467**	-0.63165 5977	no	0.4239 25135
ITPR1	ENSP00000 306253	Ca	23	0.01510 186	0.30312 0385	**Up**	**0.04511 9675**	0.339 227	**Up**	**0.00210 8493**	0.92893 3616	no	0.1193 91173
ITPR2	ENSP00000 370744	Ca	18	0.00867 089	-0.06506 6639	no	0.37017 8882	-0.187 33	**Down**	**0.00038 4716**	-0.19287 095	no	0.7101 05482
ITPR3	ENSP00000 363435	Ca	22	**0.01942 364**	-0.17880 3357	no	0.26962 3007	0.030 351	no	0.67187 5922	0.16791 1281	no	0.7425 62621
JARID 2	ENSP00000 341280	ST	1	0	-0.09663 9831	no	0.45183 5097	-0.004 31	no	0.94030 4344	-0.04113 6497	no	0.9338 51652
JUN	ENSP00000 360266	CON	31	**0.03792 962**	-0.73520 0966	**Down**	**8.92807 E-06**	-0.120 43	no	0.17136 6276	-0.06097 7387	no	0.9006 56682
KAT2 B	ENSP00000 263754	CON	18	0.00631 839	0.32267 8008	no	0.25347 3925	0.044 638	no	0.64952 4223	0.14035 1516	no	0.7297 77227
KAT6 A	ENSP00000 265713	ST	3	0.00000 55	0.08028 6036	no	0.40675 0049	-0.045 08	no	0.42703 2116	0.73482 4535	no	0.1371 27411
KIT	ENSP00000 288135	ST	20	0.00142 449	-1.50525 054	**Down**	**8.62263 E-05**	-0.625	**Down**	**8.21928 E-07**	-1.86771 7131	**Down**	**0.0166 38631**
KLF4	ENSP00000 363804	ST	8	0.00119 229	-2.19496 5499	**Down**	**1.09919 E-15**	-0.634 34	**Down**	**3.76663 E-16**	-1.72802 665	**Down**	**0.0015 27922**
LEF1	ENSP00000 265165	ST	13	0.00274 525	0.26930 9477	no	0.28355 3227	0.054 432	no	0.51193 8812	1.08833 862	no	0.0595 88598
LEFTY 2	ENSP00000 355785	ST	2	0.00035 2	0.00399 2401	no	0.94881 0225	0.032 553	no	0.54965 2823	-0.01315 8874	no	0.9771 99848
LHCG R	ENSP00000 294954	Ca	24	0.00077 5	0.01738 6812	no	0.65479 1523	0.090 285	no	0.08123 7611	-0.00207 7043	no	0.9934 90415
LIF	ENSP00000 249075	ST	4	0.00031	0.44528 7786	**Up**	**0.01453 9159**	0.341 029	**Up**	**0.00279 8926**	0.40616 1416	no	0.5813 72306
LIFR	ENSP00000 263409	ST	4	0.00027 7	0.03909 4127	no	0.40577 9435	0.038 375	no	0.48836 1884	0.67379 2536	no	0.3868 80495
LTB4R 2	ENSP00000 433290	Ca	**43**	0.00168 384	-0.44501 1631	**Down**	**1.87791 E-05**	-0.199 05	**Down**	**4.19141 E-05**	-0.37115 823	no	0.1204 65774
LYN	ENSP00000 428924	ST	26	0.00509 551	0.13056 2316	no	0.67144 0616	0.094 922	no	0.27570 6268	0.80885 1305	no	0.1317 19757
MAOA	ENSP00000 340684	CON	2	0.00682 594	-0.39519 2052	**Down**	**0.00023 0297**	-0.313 48	**Down**	**2.39977 E-05**	-0.75622 3586	no	0.3087 84189
MAP3 K1	ENSP00000 382423	ST	17	0.00469 399	0.04527 6909	no	0.68847 5865	-0.064 62	no	0.20497 2277	0.97300 1685	**Up**	**0.0097 13086**
MAPK 1	ENSP00000 215832	ST	**42**	**0.05561 423**	0.59174 0009	**Up**	**0.00041 2172**	0.039 168	no	0.42909 1242	-0.21478 692	no	0.6084 60569
MAPK 11	ENSP00000 333685	ST	17	0.00162 874	-0.13062 4401	**Down**	**0.01599 2669**	0.014 487	no	0.90517 3664	-0.71987 1659	no	0.0558 27264

MAPK 12	ENSP00000 215659	ST	14	0.00075 8	0.07470 2443	no	0.64351 3785	0.006 123	no	0.93338 8371	0.37963 7666	no	0.3060 37969
MAPK 13	ENSP00000 211287	ST	8	0.00000 825	-0.79518 4994	**Down**	**7.35263 E-10**	-0.446 36	**Down**	**4.3647 E-12**	-1.27670 5319	no	0.0972 17754
MAPK 14	ENSP00000 229794	ST	34	**0.02912 488**	0.05481 9658	no	0.60573 8821	0.116 615	**Up**	**0.03864 2425**	0.33678 4279	no	0.3587 66287
MAPK 3	ENSP00000 263025	ST	33	**0.02244 545**	-0.29981 3742	**Down**	**1.10052 E-05**	-0.087 53	**Down**	**0.01074 679**	-0.00026 5318	no	0.9993 19565
MEIS1	ENSP00000 272369	ST	1	0	-0.00032 52	no	0.99734 1657	0.015 894	no	0.68243 7074	0.19552 1186	no	0.6682 21819
MSC	ENSP00000 321445	ST	2	0	-0.44184 409	**Down**	**0.00210 32**	-0.310 33	**Down**	**5.89108 E-05**	-0.98068 8618	no	0.0907 54547
MYC	ENSP00000 367207	ST	34	**0.03598 441**	-0.13265 4894	no	0.60283 6201	-0.087	no	0.27405 7104	0.40891 0137	no	0.4496 15647
MYLK	ENSP00000 353452	Ca	6	0.00004 33	-0.57998 5987	**Down**	**0.00290 0157**	-0.184 44	**Down**	**0.00376 6651**	0.78275 8251	no	0.2371 82346
NANOG	ENSP00000 229307	ST	9	0.00072 3	-0.00755 1062	no	0.91740 2394	0.057 34	no	0.43313 4813	-0.52497 9813	no	0.3254 31383
NANOGP1	ENSP00000 432545	ST	1	0	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*
NCOR 1	ENSP00000 268712	CON	21	**0.02875 081**	-0.05988 9718	no	0.62735 1808	-0.035 62	no	0.25784 9878	0.46059 8803	no	0.1488 31132
NEUROG1	ENSP00000 317580	ST	2	0	-0.14591 6242	**Down**	**0.01067 1931**	-0.101 19	**Down**	**0.00951 7992**	-0.11084 719	no	0.4517 17158
NHP2	ENSP00000 274606	CON	2	0.00118 97	-0.07640 9985	no	0.51159 9706	-0.032 39	no	0.34746 9468	0.00238 762	no	0.9949 0292
NODAL	ENSP00000 287139	ST	6	0.00104 798	-0.04888 8979	no	0.60238 1853	0.115 13	**Up**	**0.02397 9605**	-0.13189 2887	no	0.5698 11133
NOS1	ENSP00000 337459	Ca	32	0.01553 999	-0.07765 8425	no	0.15655 9391	-0.117 54	no	0.14979 999	-0.70128 5144	no	0.1223 62533
NOS2	ENSP00000 327251	Ca	8	0.00074 9	-0.14896 1943	**Down**	**0.01591 3971**	-0.083 47	**Down**	**0.00745 4467**	-0.19224 5648	no	0.4144 33232
NOS3	ENSP00000 297494	Ca	18	0.00431 314	0.12962 4134	no	0.17199 9398	0.103 497	**Up**	**0.00751 6629**	0.04351 4375	no	0.8835 64244
NRAS	ENSP00000 358548	ST	27	0.00308 327	0.80228 4309	**Up**	**4.36087 E-06**	0.230 293	**Up**	**0.00014 7968**	0.10351 2882	no	0.7544 82623
NTSR1	ENSP00000 359532	Ca	**42**	0.00000 382	-0.01293 9618	no	0.84570 0513	-0.049 78	no	0.48948 4169	-0.45939 4248	no	0.3593 47726
ONECUT1	ENSP00000 302630	ST	2	0	0.00374 5174	no	0.96230 6229	0.000 58	no	0.99577 601	-0.12716 7075	no	0.4812 34016
ORAI1	ENSP00000 328216	Ca	6	0.00675 581	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	-0.20995 0928	no	0.5736 66687
ORAI2	ENSP00000 348752	Ca	2	0	0.06198 5934	no	0.39732 3384	0.038 541	no	0.61249 3991	1.41878 4624	no	0.0855 57761
ORAI3	ENSP00000 322249	Ca	3	0	0.04162 2657	no	0.70884 7963	-0.024 18	no	0.49090 4569	0.33913 4672	no	0.3145 46818
OTX1	ENSP00000 282549	ST	1	0	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	-0.17714 2623	no	0.2355 25365
OXTR	ENSP00000 324270	Ca	**46**	0.00449 222	-0.00710 418	no	0.92995 1657	0.007 858	no	0.82289 6304	0.24470 0775	no	0.4927 95142
P2RX1	ENSP00000	Ca	1	0	-	no	0.36165	0.058	no	0.08216	-	no	0.6512

	225538				0.066747191		847	28		0849	0.1585924		679
P2RX2	ENSP00000343339	Ca	3	0.0136285	-0.069609197	no	0.294649184	-0.00956	no	0.818396972	-0.433467644	**Down**	**0.042320371**
P2RX3	ENSP00000263314	Ca	1	0	-0.016075485	no	0.766270034	0.097248	no	0.057139593	-0.03548392	no	0.841640553
P2RX4	ENSP00000336607	Ca	1	0	0.412726095	**Up**	**0.005131297**	0.096306	**Up**	**0.00916174**	0.23700254	no	0.609447321
P2RX6	ENSP00000416193	Ca	1	0	-0.114025437	no	0.080183886	0.145606	**Up**	**0.000205534**	-0.0778463	no	0.721910798
P2RX7	ENSP00000442349	Ca	1	0	-0.556898902	**Down**	**0.009570002**	-0.08705	no	0.077134747	-0.013761729	no	0.985812605
PAX6	ENSP00000368401	ST	7	0.01430283	-0.023637145	no	0.815807945	-0.04449	no	0.555753933	-0.123458766	no	0.832131827
PDGFRA	ENSP00000257290	Ca	19	0.00114609	-0.799235614	**Down**	**0.000702045**	-0.02633	no	0.629107468	0.688206632	no	0.255263685
PDGFRB	ENSP00000261799	Ca	24	0.00281377	-0.572694082	**Down**	**9.96889E-05**	-0.32633	**Down**	**1.79765E-06**	0.066832333	no	0.898623877
PHKG1	ENSP00000297373	Ca	2	0	-0.08593137	no	0.210806349	0.05123	no	0.146889631	-0.080881259	no	0.639821948
PIK3CG	ENSP00000352121	ST	35	0.0053544	0.131659975	no	0.127934481	-0.11271	no	0.211988872	0.323612116	no	0.62727963
PIK3R1	ENSP00000274335	ST	**69**	**0.05441236**	-0.176014314	no	0.383906559	-0.12078	**Down**	**0.030059149**	0.834032706	no	0.057825554
PIK3R5	ENSP00000392812	ST	33	0.00494904	-0.025525624	no	0.719392022	-0.00378	no	0.901719996	-0.813811885	no	0.10381709
PLCB1	ENSP00000338185	Ca	**68**	0.01829564	0.087306204	no	0.337552618	0.109468	no	0.159596073	-0.492895971	no	0.444877184
PLCB2	ENSP00000260402	Ca	**66**	0.01380784	-0.047274128	no	0.509178203	-0.09286	no	0.109728175	-0.060798796	no	0.893980024
PLCB3	ENSP00000279230	Ca	**66**	0.01380784	-0.333402195	**Down**	**2.05604E-05**	-0.00961	no	0.850449402	-0.481368338	**Down**	**0.02030294**
PLCB4	ENSP00000334105	Ca	**66**	0.01320147	0.109592421	no	0.384080228	0.079701	no	0.452082054	0.123290515	no	0.92354817
PLCD1	ENSP00000430344	Ca	18	0.000326	-0.320143416	**Down**	**8.46033E-08**	-0.06417	no	0.169646016	-0.635162508	no	0.108759044
PLCD3	ENSP00000313731	Ca	17	0.000153	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	-0.612677715	**Down**	**0.004968125**
PLCD4	ENSP00000388631	Ca	17	0.000153	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	-1.151376888	**Down**	**0.016222404**
PLCE1	ENSP00000260766	Ca	21	0.000526	0.004084141	no	0.958541755	0.041715	no	0.475458787	0.898823901	no	0.178537484
PLCG1	ENSP00000244007	Ca	**49**	0.01645968	0.034969043	no	0.747405232	0.036399	no	0.38789858	0.555452063	**Up**	**0.024680198**
PLN	ENSP00000350132	Ca	4	0.00144688	-0.047958773	no	0.74195927	0.02075	no	0.801210628	0.795372779	no	0.295586562
POU5F1	ENSP00000259915	ST	7	0.00066	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*
PPIF	ENSP00000225174	Ca	6	0.01127904	0.191873255	no	0.198210866	0.082622	no	0.213416041	0.731576058	no	0.197484379
PPP1CC	ENSP00000335084	CON	22	**0.01965509**	0.609747304	**Up**	**1.52233E-05**	0.157544	**Up**	**3.22551E-05**	0.167375812	no	0.307997432

PRC1	ENSP00000377793	ST	3	0	0.605005497	**Up**	**0.004295023**	0.142992	**Up**	**0.009293472**	0.13587168	no	0.728506463
PRKACA	ENSP00000309591	CON	**51**	**0.0837037**	-0.229739375	**Down**	**0.00193898**	-0.10236	**Down**	**0.006166758**	0.577944363	no	0.056947292
PRKCA	ENSP00000408695	Ca	**55**	**0.03906664**	0.476902328	**Up**	**0.003860994**	-0.0969	no	0.227826787	-0.048769984	no	0.934220679
PRKCB	ENSP00000305355	Ca	**44**	0.0169036	0.322588256	no	0.314271066	-0.03037	no	0.600954741	0.286916522	no	0.754843222
PRKCG	ENSP00000263431	Ca	35	0.0071681	-0.111048826	no	0.102029504	-0.08034	no	0.238398963	-0.165918417	no	0.355109799
PROM1	ENSP00000415481	ST	1	0	0.060273534	no	0.315180126	0.094452	**Up**	**0.015040622**	0.262971426	no	0.783265559
PTAFR	ENSP00000301974	Ca	**43**	0.00208304	-0.011675406	no	0.925457757	-0.16196	**Down**	**0.012312197**	-0.303528499	no	0.542361345
PTEN	ENSP00000361021	ST	22	0.00613556	0.37856366	**Up**	**0.004000411**	0.074292	no	0.235543 71	-0.076317161	no	0.865182847
PTGER1	ENSP00000292513	Ca	**45**	0.000485	-0.216545773	**Down**	**0.000063034**	-0.06608	no	0.476587127	-0.220067713	no	0.339085974
PTGER3	ENSP00000349003	Ca	15	0.000279	-0.01796286	no	0.794789452	0.214549	**Up**	**0.001120931**	-1.465898247	**Down**	**0.042834648**
PTGFR	ENSP00000359793	Ca	**42**	0.00000382	-0.173437897	**Down**	**0.011621634**	-0.09078	**Down**	**0.049374287**	-0.734212216	no	0.104475344
PTK2B	ENSP00000332816	Ca	20	0.00295102	-0.305563784	**Down**	**0.011750578**	-0.14236	**Down**	**0.010198577**	-0.34433873	no	0.385406587
PTPN11	ENSP00000340944	ST	34	0.02374219	0.348588259	**Up**	**0.039908343**	0.14705	**Up**	**0.009092992**	1.207936326	**Up**	**0.048264037**
RAF1	ENSP00000251849	ST	27	0.01186176	-0.122772265	no	0.248641936	-0.07943	**Down**	**0.00124894**	0.559790317	no	0.183138052
REST	ENSP00000311816	ST	2	0	-0.097581434	no	0.243497937	0.254397	**Up**	**0.000310556**	1.009594871	**Up**	**0.024198881**
RIF1	ENSP00000243326	ST	1	0	-0.003397582	no	0.942112086	0.03343	no	0.688044015	1.500650292	**Up**	**0.038049024**
RRAS	ENSP00000246792	ST	12	0.000282	-0.377633449	**Down**	**0.000603199**	-0.16525	**Down**	**5.35327E-05**	-0.482769566	no	0.289382644
RYR1	ENSP00000352608	Ca	7	0.000699	-0.469446952	**Down**	**0.01358497**	0.004308	no	0.948027757	-0.262756533	no	0.733136393
RYR2	ENSP00000355533	Ca	10	0.000959	-0.076448117	no	0.152839664	0.217964	**Up**	**0.000231604**	-0.347690769	no	0.668423945
RYR3	ENSP00000373884	Ca	4	0.0000905	0.042931871	no	0.340210558	0.016311	no	0.601497128	-1.045794583	**Down**	**0.035007429**
SALL4	ENSP00000217086	CON	10	0.00803744	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	*no probe*	0.414972234	no	0.310725094
SDC1	ENSP00000254351	CON	7	0.00959994	-1.517841904	**Down**	**3.24338E-08**	-0.88533	**Down**	**1.98433E-18**	-1.209817907	no	0.120751119
SETDB1	ENSP00000271640	ST	4	0.00687492	-0.082886696	no	0.388790347	-0.00268	no	0.956762155	0.347017789	no	0.33229295
SKIL	ENSP00000259119	ST	7	0.0000762	0.080206068	no	0.184974683	0.243431	**Up**	**0.000961394**	1.217523077	**Up**	**0.042111939**
SLC25A4	ENSP00000281456	Ca	4	0	-0.070555962	no	0.758605509	-0.03586	no	0.509293775	-0.173522016	no	0.753328025
SMAD2	ENSP00000262160	ST	29	**0.02336588**	0.210662187	**Up**	**0.009466433**	-0.005	no	0.924719732	-0.35259	no	0.23296179

								93			4394		
SMAD 3	ENSP00000 332973	ST	28	**0.02392 531**	0.00473 7328	no	0.97564 7588	0.107 429	no	0.09794 078	- 0.38990 1573	no	0.2198 62918
SMAD 4	ENSP00000 341551	ST	35	**0.03753 624**	0.79137 4313	**Up**	**5.56315 E-06**	0.169 068	**Up**	**0.00029 2094**	0.76765 0726	no	0.1414 17242
SMAD 6	ENSP00000 288840	ST	11	0.00006 36	- 0.17530 8617	**Do wn**	**0.00352 4164**	- 0.204 26	**Do wn**	**0.01156 6706**	- 0.25831 4473	no	0.6137 23435
SMAD 7	ENSP00000 262158	ST	23	0.01209 405	- 0.02819 4174	no	0.80415 7049	- 0.081 13	no	0.09154 8427	0.27688 9098	no	0.4758 79695
SMAR CAD1	ENSP00000 351947	ST	1	0	*no probe*	*no pr ob e*	*no probe*	*no prob e*	*no pro be*	*no probe*	0.44053 724	no	0.1062 29561
SOX10	ENSP00000 354130	CON	4	0.00714 344	0.10563 2401	no	0.74656 7393	0.004 321	no	0.97040 4437	0.47176 8938	no	0.4652 72466
SOX2	ENSP00000 323588	ST	11	0.00285 805	- 0.02605 9678	no	0.75819 2096	- 0.137 04	no	0.05585 6357	- 1.42420 7065	no	0.2052 19819
SPHK 1	ENSP00000 313681	Ca	8	0.00092 8	0.33204 8987	**Up**	**0.02205 5475**	0.069 38	no	0.19248 5996	- 0.66647 5986	no	0.2001 80758
STAT3	ENSP00000 264657	ST	28	0.01545 67	- 0.02889 9098	no	0.83452 0123	- 0.096 68	no	0.05432 4093	0.78393 5787	no	0.0586 44045
STIM1	ENSP00000 300737	Ca	7	0.01353 499	- 0.24465 3986	**Do wn**	**0.00048 8812**	- 0.038 82	no	0.20212 5033	0.16112 7766	no	0.6957 34504
STIM2	ENSP00000 417569	Ca	4	0.00002 34	*no probe*	*no pr ob e*	*no probe*	*no prob e*	*no pro be*	*no probe*	0.69087 6505	no	0.1411 03889
TACR 1	ENSP00000 303522	Ca	**42**	0.00000 382	- 0.12607 6241	no	0.12428 7544	- 0.078 38	no	0.14092 9089	- 1.60748 9836	**Do wn**	**0.0069 47436**
TACR 2	ENSP00000 362403	Ca	**42**	0.00000 382	- 0.09373 2789	no	0.18858 5427	0.061 091	no	0.20853 2278	0.25173 582	no	0.4623 3114
TACR 3	ENSP00000 303325	Ca	**42**	0.00000 382	0.09407 3756	no	0.19629 4259	0.037 577	no	0.20630 9235	- 0.01905 164	no	0.9021 23229
TBX3	ENSP00000 257566	ST	1	0	0.15667 443	no	0.23086 8791	0.087 755	no	0.46113 262	1.01426 1146	no	0.0909 3181
TBXA2 R	ENSP00000 393333	Ca	**47**	0.00301 697	- 0.09454 9545	no	0.14230 7589	0.118 784	**Up**	**0.00364 0564**	0.36666 9553	no	0.5449 11813
TCF3	ENSP00000 262965	ST	18	**0.02573 384**	0.03089 2143	no	0.80348 1248	0.118 718	no	0.21713 9092	0.38819 6991	no	0.2601 91349
THY1	ENSP00000 284240	ST	1	0	- 0.47345 1414	**Do wn**	**0.00038 2645**	- 0.251 12	**Do wn**	**9.17744 E-05**	- 0.09768 9432	no	0.8675 12422
TLE4	ENSP00000 365735	CON	5	0.00682 854	0.13059 6856	no	0.44965 7637	0.017 118	no	0.85236 3111	0.11219 1689	no	0.8755 86586
TRHR	ENSP00000 309818	Ca	**42**	0.00000 382	0.13902 5291	**Up**	**0.04514 731**	0.110 461	**Up**	**0.00024 0935**	- 0.14019 9544	no	0.4424 0227
VDAC 1	ENSP00000 265333	Ca	7	0.01610 604	0.31425 0026	**Up**	**0.01885 6803**	- 0.004 27	no	0.94619 8996	0.92412 0346	no	0.0924 89525
VDAC 2	ENSP00000 361635	Ca	4	0	0.10089 7002	no	0.47805 0986	- 0.022 76	no	0.62051 6463	- 0.27889 1688	no	0.1432 31604
VDAC 3	ENSP00000 428845	Ca	5	0.00682 594	0.12215 0714	no	0.31336 3466	0.040 102	no	0.30587 8668	1.00234 3479	no	0.0602 85107
WNT1	ENSP00000 293549	ST	33	0.01609 896	- 0.00444 5517	no	0.94702 7658	0.052 91	no	0.13890 4226	- 0.14643 2726	no	0.4202 89685
WNT1 0A	ENSP00000 258411	ST	27	0.00002 01	*no probe*	*no pr ob e*	*no probe*	*no prob e*	*no pro be*	*no probe*	- 1.05101 4506	**Do wn**	**0.0061 04193**
WNT1	ENSP00000	ST	28	0.00423	-	no	0.54975	-	no	0.17291	-	no	0.2164

0B	301061			025	0.06527 3366		1248	0.102 01		3743	0.45959 241		14778
WNT1 1	ENSP00000 325526	ST	27	0.00002 01	- 0.19877 9226	**Do wn**	**0.00223 7576**	- 0.160 37	**Do wn**	**0.02908 149**	- 0.60112 241	no	0.1265 76731
WNT1 6	ENSP00000 222462	ST	27	0.00002 01	0.01744 3978	no	0.79630 8339	0.057 444	no	0.23734 5562	- 2.13889 0939	**Do wn**	**0.0149 76208**
WNT2	ENSP00000 265441	ST	28	0.00077 4	0.14920 1682	no	0.07729 9817	0.103 869	**Up**	**0.00850 0292**	- 0.20696 4908	no	0.6597 177
WNT2 B	ENSP00000 358698	ST	27	0.00002 01	- 0.05721 198	no	0.39377 9725	0.012 966	no	0.79800 7509	- 0.29092 1771	no	0.4047 09278
WNT3	ENSP00000 225512	ST	27	0.00002 01	- 0.34589 1879	**Do wn**	**9.14835 E-07**	- 0.085 56	**Do wn**	**0.00537 9583**	- 0.62360 6598	no	0.3563 00932
WNT3 A	ENSP00000 284523	ST	33	0.01580 006	*no probe*	*no pro be*	*no probe*	*no prob e*	*no pro be*	*no probe*	*no probe*	*no pro be*	*no probe*
WNT4	ENSP00000 290167	ST	27	0.00002 01	- 1.03378 4268	**Do wn**	**1.73126 E-15**	- 0.408 09	**Do wn**	**7.82447 E-15**	- 1.47788 7308	**Do wn**	**0.0177 02159**
WNT5 A	ENSP00000 264634	ST	29	0.00866 196	- 0.62506 5857	**Do wn**	**2.03354 E-06**	- 0.228 63	**Do wn**	**4.43848 E-06**	- 1.66925 5786	**Do wn**	**0.0017 85341**
WNT5 B	ENSP00000 308887	ST	27	0.00002 01	- 0.19662 1821	**Do wn**	**0.01713 064**	- 0.109 38	no	0.05840 3641	- 0.77866 6315	no	0.1165 73098
WNT6	ENSP00000 233948	ST	27	0.00002 01	0.08957 4217	no	0.32399 6843	- 0.094 6	**Do wn**	**0.03067 9811**	- 0.59501 5336	no	0.1286 00012
WNT7 A	ENSP00000 285018	ST	28	0.00077 4	- 0.07991 7014	no	0.11679 2267	- 0.019 28	no	0.68929 0889	- 0.35579 0357	no	0.1050 68669
WNT7 B	ENSP00000 341032	ST	27	0.00002 01	- 0.31256 1559	**Do wn**	**4.87495 E-06**	- 0.195 48	**Do wn**	**5.79645 E-06**	- 0.46383 41	**Do wn**	**0.0331 64429**
WNT8 A	ENSP00000 381739	ST	30	0.00310 753	*no probe*	*no pro be*	*no probe*	*no prob e*	*no pro be*	*no probe*	- 0.19547 4245	no	0.3140 72573
WNT8 B	ENSP00000 340677	ST	30	0.00310 753	- 0.11899 919	no	0.08635 3151	- 0.018 3	no	0.77882 8694	- 0.07904 5592	no	0.6858 34465
WNT9 A	ENSP00000 272164	ST	27	0.00002 01	*no probe*	*no pro be*	*no probe*	*no prob e*	*no pro be*	*no probe*	- 0.79681 0057	**Do wn**	**0.0172 24254**
WNT9 B	ENSP00000 290015	ST	27	0.00002 01	*no probe*	*no pro be*	*no probe*	*no prob e*	*no pro be*	*no probe*	- 0.06612 0029	no	0.6969 58394
ZIC3	ENSP00000 287538	ST	5	0	0.12027 8857	no	0.06559 5744	0.151 457	**Up**	**0.00949 0133**	- 0.21748 7605	no	0.2032 26624
ZNF59 3	ENSP00000 363384	ST	2	0.00016 4	- 0.08258 3463	no	0.60832 8638	0.001 154	no	0.98499 5034	0.42242 9503	no	0.4416 91105

3. Discussão dos Resultados

No artigo do presente trabalho, apresentamos a montagem de um grafo modelando a interação entre proteínas membros da integração das vias de sinalização do íon cálcio e da regulação da pluripotência de células tronco. A rede apresenta alta densidade: 2995 interações para 294 proteínas, com valor de enriquecimento de interações significativo (teste hipergeométrico com $p<0,001$). Isso indica que essas proteínas tem maior grau de conectividade do que esperado ao acaso para um conjunto de nós do mesmo tamanho e isso, por consequência, sugere uma ligação biológica modular entre elas. Esse resultado corrobora com o fato de que as proteínas vieram de anotações de vias anotadas pelo KEGG, portanto se espera que sejam funcionalmente ligadas em nível de interatoma. O algoritmo proposto conectou as proteínas de ambas as vias analisadas sem perder o enriquecimento significativo de interações e gerando um modelo *in silico* robusto. Além disso, embora o limiar para o escore de confidência das interações tenha sido de 0,400 (de média confiança), os escores das interações de toda a rede apresentam média > 0,864 (alta confiança), reforçando a robustez deste modelo. Por fim, a rede segue uma distribiuição de graus livre de escala com α igual a aproximadamente 7 (teste de Kolmogorov-Smirnov com valor $p>0,05$, rejeitando a hipótese de que a distribuição de graus não segue uma lei exponencial). Isso mais uma vez reforça a validade do modelo, pois sabe-se que grafos oriundos de redes biológicas têm como propriedade quase universal a distribuição livre de escala (JEONG *et. al.*, 2000). Este modelo recebeu o nome de *STEMCa* e será referenciado assim daqui adiante no texto.

Em uma segunda etapa, utilizamos experimentos independentes de grupos de pacientes com melanoma, para os quais foram feitas biópsias de tumores primários ou metastáticos, e essas amostras foram submetidas a semi-quantificação da expressão gênica por microarranjo. Esses dados foram utilizados para análise de expressão diferencial, a qual

apontou que mais de 50% (151 de 294) dos genes que codificam as proteínas membros do modelo *STEMCa* estão diferencialmente expressos (valor p corrigido por FDR < 0.05) em pelo menos um dos experimentos avaliados. Isso sugere que, a nível transcricional, a integração das vias de sinalização do cálcio e regulação da pluripotência de células tronco está desregulada nas células com poder de metástase. Dentre os 151 genes diferencialmente expressos, 64 pertencem à via de sinalização do cálcio, enquanto 75 pertencem à via de regulaçao da pluripotência de células tronco, portanto são ambas as vias que contribuem para a expressão aberrante no modelo "STEMCa". Além disso, o algoritmo utilizado para montagem e integração da rede adicionou 21 nós (proteínas) não anotadas pelo KEGG como pertencentes à nenhuma das vias avaliadas, os quais são chamados nesse trabalho de *conectores*. Dos 21 *conectores* adicionados ao modelo "STEMCa", 11 estão diferencialmente expressos, dentre os quais 7 ocupam posições centrais no interatoma (alto grau e/ou alto *betweenness*). Isso sugere que a integração dos *conectores* é funcional (com significado biológico) e não ao acaso, pois eles contribuem tanto para o perfil modular do interatoma (*i.e.* sua remoção levaria ao colapso da rede) quanto para a expressão desregulada de ambas as vias estudadas.

Contudo, a análise de expressão diferencial limita-se à avaliação individual do status de cada gene (super ou subexpresso), ao passo que nosso estudo busca também a avaliação da expressão gênica em metástase de melanomas para o modelo "STEMCa" como um conjunto modular único. Para atingir esse objetivo, utilizamos a ferramenta *GALANT*, a qual plota perfis de expressão gênica sobre redes de interação proteica com base no *log Fold Change* dos genes codificadores das proteínas que compõe a rede estudada. Com o resultado apresentado na figura 2 do artigo, fica evidente a similaridade consistente entre os perfis de expressão do modelo "STEMCa" nos 3 conjuntos de dados indepentes que foram avaliados.

Por fim, os cálculos topológicos feitos sob a rede apontam as proteínas *CTNNB1*, *GNAQ*, *GSK3B*, *GSTP1*, *MAPK3*, *PPP1CC*, *PRKACA*, *SMAD4*, e *PTPN11* como aquelas que têm posição central no interatoma e ao mesmo tempo se apresentam diferencialmente expressas. Ou seja, esse subconjunto de proteínas do modelo "STEMCa" exerce forte influência dentro interatoma por conta de suas propriedades topológicas e, portanto, tendo a expressão de seus genes codificadores desregulada, deverão ifluenciar no funcionamento modular da rede como um todo (análogo a se estragar uma peça fundamental dentro de um motor, levando-o ao mal funcionamento). Por essa razão, essas proteínas centrais são aqui sugeridas como potenciais candidados de um grupo biomarcador de metástase em melanomas, assim como possíveis alvos terapêutipos.

A detecção de metástases nos estágios iniciais é difícil e, por isso, a migração de células tumorais para tecidos além do sítio do tumor representa mais de 90% das mortes causadas por câncer (Chambers *et al.*, 2002). Sendo assim, a elucidação das vias que atuam na promoção de metástase e a exploração de novos biomarcadores que contribuam para a detecção precoce de uma neoplasia potencialmente maligna são de grande relevância.

Nosso modelo "STEMCa" busca modelar a integração das vias de regulação do íon cálcio e da regulação da plutipotência de CTs. Sabe-se que as CTs e as CTTs dividem características similares em nível celular (potência e auto-renovação) e molecular (expressão de marcadores como, por exemplo, proteínas de superfície e biomarcadores de pluripotência) (Oren e Smith, 2016). As CTTs, por sua vez, apresentam características importantes para promover metástase em câncer, já que possuem o poder de auto-renovação e também podem dar origem às demais células que compõe a população heterogênea do tumor. A literatura científica é vasta de exemplos demonstrando que células de um tumor que expressam marcadores de tronco tendem a ser mais invasivas e, mesmo em pequena quantidade, são capazes de repopular todo o sítio tumoral, restaurando o câncer (Jordan e Guzman, 2004;

Singh, Clarke, *et al.*, 2004; Valk-Lingbeek *et al.*, 2004). O segundo componente do modelo "STEMCa" refere-se à sinalização dependente do íon cálcio, a qual tem papel fundamental na proliferação e diferenciação celular em CTs no estágio embrionário (Carafoli e Krebs, 2016). Apesar da falta de estudos detalhados das vias de sinalização de Ca^{2+} em CTs pluripotentes, nós especulamos que a atividade transcricional de ambos componentes do modelo "STEMCa" pudessem contribuir para a promoção de metástase em melanomas. Nossos resultados sugerem que essa hipótese é válida, embora estudos seguintes sejam necessários para confirmação destes dados em nível proteico.

Outro processo que parece relevante para a progressão de metástases é a transição epitélio-mesenquimal (TEM), que embora tenha papel fundamental no desenvolvimento da mesoderme nos estágios de desenvolvimento embrionário, também é reconhecida como um mecanismo patológico no surgimento de várias doenças, incluindo fibrose e câncer (Gos *et al.*, 2009). Sabe-se que durante a TEM, uma célula com atributos fenotípicos de células epiteliais sofre uma radical reprogramação celular, passando a ter atributos de células mesenquimais (*i.e.* tornam-se móveis e capazes de invadir a matriz extra-celular), ou seja, porencialmente mais invasivas (Lee *et al.*, 2006). No modelo *STEMCa* temos um clique de proteínas da via de sinalização *Wnt* com expressão aberrante, para qual estudos sugerem que sua ativação contribui para a indução e manutenção de CTTs ativadas por TEM (Scheel *et al.*, 2011). Apresentamos ainda *CTNNB1* como um dos nós centrais da rede, uma proteína que provê o recrutamento de fatores de transcrição ativados pela via de sinalização *Wnt* (Chang *et al.*, 2015). Além disso, observamos GSK3B (cinase que reprime a via de sinalização *Wnt*, fosforilando CTNNB e, assim, marcando-a para degradação) como um nó gargalo e significativamente subexpresso em 2 das 3 côrtes avaliadas.

As fosfatases têm poder de controlar amplitude e intensidade de sinais de cascatas de fosforilação, que exercem papel fundamental em muitos processos biológicos, dentre os

quais está o comprometimento entre proliferação/diferenciação (Tonks, 2013). Com isso, apontamos outra proteína pertencente ao grupo central do modelo *STEMCa*: a PPP1CC, que pertence à família de fosfatases, subfamília PP1. Takarura *et. al.* avaliou sistematicamente o papel de 9 fosfatases da família PP1 (incluindo PPP1CC) em 55 linhagens celulares de cânceres humanos, abrangendo pulmão, coloretal, gástrico e ovário (Takakura *et al.*, 2001). Apesar da expressão constitutiva de muitos dos genes avaliados em todas as linhagens, foram observadas alterações puntuais de expressão diferencial e mutações somáticas e/ou polimorfismos câncer-específicos em membros da família PP1, indicando seu envolvimento na carcinogênese. Além disso, PPP1CC já foi reportada em outro modelo de interação proteína-proteína como um nó central em câncer coloretal (Kou *et al.*, 2015), tendo sido este modelo criado a partir de genes desregulados em amostras de biópsia de tumores em comparação a tecidos saudáveis. Em especial, no entanto, apontamos a fosfatase PTPN11, da família de proteínas tirosina-fosfatases, como a única que mostrou constituitivamente superexpressão nos 3 conjuntos de dados independentes avaliados. Estudos mostram que a inibição de PTPN11 impede a aquisição de quimioresistência a drogas que funcionam ativando receptores de tirosina cinase. Sabendo-se que as CTTs apresentam quimioresistência, a superexpressão consistente de PTPN11 e sua centralidade no modelo "STEMCa" sugerem esse gene/proteína como um biomarcador interessante a ser investigado.

Por último, conclui-se nesse trabalho:

1. Obtivemos um modelo de interação proteína-proteína robusto da integração da via de sinalização do íon cálcio e regulação da pluripotência de CTs.
2. Observamos um padrão consistente de expressão anormal do perfil transcricional dos genes codificadores das proteínas membro do modelo

proposto ao se comparar tumores primários e metastáticos de melanoma humano.

3. Identificamos e propomos, através da combinação de cálculos topológicos e expressão diferencial, um conjunto de genes biomarcadores de metástase em melanoma humano, com destaque à fosfatase PTPN11.

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