Genome-Wide Analysis of The ID Family of Bhlh TF's In Glial Tumours

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Research Article

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Abstract

Background: The inhibitor of differentiation (ID) family of TF's accumulated the result of development. This subgroup of bHLH TF's is an inverse regulator that acquires to constrain segregation and stimulate proliferation. The ID family of bHLH TF's control the reactions of homodimer and heterodimer by motions of E proteins (Class A) and tissue-specific (Class B) bHLH domain. A recent report suggested ID genes act to enhance the proliferative potential of tumour astrocytes. Those reports supported ID genes are mighty regulators in tumour-angiogenesis and govern the malignant response of glial tumours. So, I performed bioinformatics and computational application to the current knowledge of the ID family in two different genomes.

Results: My finding supported the number of ID1-ID4 genes and their encoded proteins present in two isolated organisms. Therefore, I documented the molecular functions and mechanisms linked with the ID family of TF's in mammals. Those mechanisms assume the ID1-ID4 of bHLH domains reveals an inherent role during differentiation, cell-cycle regulation, and cellular growth.

Conclusion: My finding data provided the foundation of ID1-ID4 genes in glial tumours. Also, the numerous molecular mechanisms described the hallmark of glial development.

Highlights:

- The P53 is a repressor of ID1/ID2 functions and cellular proliferation.
- ID2 with RB differentially repress G1/S and G2/M associated genes after P53 activations.
- CDKIs are suppressors of p53 and pRB, and E2F-1, all degrade by ubiquitin/proteasome.
- ID2 activate by the enlightenment of chimeric proteins.
- ID2/ID4 inhibits OLIG1/OLIG2 are robust in oligodendrocyte development.
- ID2/ID3 initiation is powerful during B cells development.
- ID3 captivate immune checkpoints during T cell development.

Introduction:

The feature of neuroglia originates from the potentiality of genes to segregate the postpartum period. Glial growth characterized encephalon to respond gliosis and malignant fluctuation of neuroglia. The primary tumours in the encephalon represent astrocytic derived tumours. Astrocytes tend to the malignant transformation that differentiates cells in CNS [1–4]. The astrocytic tumour accord ubiquitous characteristic of effective astrocyte enhances genes and proteins function. Those genes and their encoded proteins oscillation induce at developmental stages of astrocytic differentiation. Known studies exhibit the viability of effective state imparts by astrocytes under the reaction of neoplastic activity. The molecular mechanisms described the salient improvement and stimulating of neuroglia also glial pathology remains unclear. But the neuroglia activation and malignant variation of astrocytes depend on

the process of ID genes and proteins [5]. The inhibitor differentiation (ID) genes that act in encephalon are optimum and limited to recover the strength of astrocytes. Despite, likelihood glial cell differentiates by the robust accumulation of ID gene and their encoded proteins in glial tumours [6]. An earlier study suggested the ID genes expressed at variable levels in cells acquire from glial tumours such as neuroblastoma, glioblastoma and glioma [1]. Recent experimental evidence supported the glial tumours obtains from the CNS also express the high degree of ID1-ID4. The vector of inhibitor differentiation examines depend on the pathological classification of the malignancy. Also, the unstable expression of ID genes suggested aggressive growth of glioblastoma multiform and astrocytoma [7]. ID genes exhibit in malignant cells and blood vessels during the enhancement of astrocytic tumours. The malformed function of ID1-ID3 in astrocytic tumours appears in blood vessels correlated by an intensity of endothelial proliferation. Abnormal function of ID1-ID3 suggested glioma, medulloblastoma and neuroblastoma. During angiogenesis, tumours specific ID1/ID3 in model organisms fail to develop and metastasize. Under these circumstances, neovascularization cruelly damages substantial regions on haemorrhage and necrosis [7–11]. Since the characteristic of tumour progression in NS is an equilibrium between anti-angiogenic and pro-angiogenic (angiogenic switch) molecules. Those molecules lead to the tumour neovascularization associated with brain tumours [12, 13]. The aggressive gliomas generally coordinated with eminent vascular proliferation required oxygen even nutrients to enhance tumour mass. Thus, neo-angiogenesis in tumours drive ID genes and proteins in the tumour endothelium. The degree of ID1-ID4 functions supported anti-angiogenic and targets against highly vascularized brain tumours. The growth of PNS/CNS reveals the interaction of E proteins (E2A, E2-2/ITF2, and HEB/HTF4) and tissue-specific (MyoD, NeuroD, MASH, TAL, and MYOG) bHLH TF's promoting a strategy of cell-fate differentiation [14, 15]. In neurogenesis, bHLH domains are key regulators that develop neuronal differentiation required OLIG1-OLIG3, NEUROG1-NEUROG3, NEUROD1, NEUROD2, NEUROD4, NEUROD6, ATOH1, ATOH7, ATOH8, and ASCL1 [16–21]. The proneural nuclear genes stimulate lineage-specific differentiation through neurogenesis and determine patterns of cellular differentiation during development [22–25]. The neurogenic factor of HES1 binds and resists the functions of the bHLH domain, which inhibits transcription and prevents neural differentiation and specification [26]. HES1 restrains neurogenic differentiation strategy and exhaust the pool of neural precursors [27–30]. The ID's binds and inhabits HES1 during the growth of the NS (nervous system). Both are inhibitors of neurogenesis and control the response of negative regulation to allow transcription of specific proneural bHLH TF's [31]. An earlier report suggested the elevated levels of ID functions rapidly induced in cells and survive through the S phase. Those studies proposed the G1 progression requires functions of E2A and ID genes in specific targets [32]. The signalling of ID1-ID4 intersects with bHLH, ETS, E2A, HEB, E2-2, PAX's, E2F and other TF's to form complex differentiation for the viability of organisms [33–36]. The ID family of bHLH TF's prevent DNA binding and control interactions of other factors. But it's unclear the inhibitor differentiation (ID) factors have a positive role in cell proliferation. A recent report supported the molecular checkpoint control proliferation by pocket proteins family or RB family (Rb, p107, and p130). Especially, ID2 bind with Rb, p107, p130 in a cell cycle when massive abundance inhibits their anti-proliferative functions [37–40]. This appearance characterized neuroectodermal tumours when ID2 molar redundancy over active hypophosphorlated RB [41]. Furthermore, the E2F family are vital for RB function,

but the functional inhabitation between the cellular RB-ID2 and RB-E2F functions are unclear. However, ID2/E2F participate RB binding, while ID2 revealed by the mobility of the RB family (pocket proteins) family depend on E2F transcription. The enormous RB-ID2 and RBL1 (p107)-ID2 complexes establish S phase quench the signal of ID2 in natural target since the comparison disputed the ID2 activity characterized G1 progression. The negative obligation of the RB family restrains ID2 activity is fundamental for the S phase and cell viability [42, 43]. Since the negative factor in growth-promoting controlled by tumour suppressor protein is vital for sustain tissue-homeostasis [1]. The inverse role of the RB family with inhibitor differentiation is necessary to quench inhibitory firing of anti-proliferation and differentiation. Also, most recent reports suggested the ID1 inhabits ETS (ETS1/ETS2) mediated transcription of p16 as a tumour suppressor factor that has a function uniform to the pocket proteins family [42]. Those factors elicit the activity of inhibitor differentiations inactivate the mobility of pocket proteins. The anti-apoptotic ability of the ID family might assign a counterforce to support full immortalization. Thus, it is striking the apoptosis promotes by elements of BCL-2/BCL-XL (anti-apoptotic) precisely enhance ID-mediated immortalization by accessing dual ability to lead cell outgrowth and death [44–46]. Inhibitor differentiations (ID1-ID4) associated with polypeptides that combine with a genus of bona fide growth-promoting proteins such as MYC and E2F1 are robust activators of apoptosis. The oncogenic action is strongly affected by the survival genes of the BCL-2/BCL-XL in the BCL-2 family [47–50]. In this work, an intense glimmer of hope and evidence justify the inclusion of the inhibitor differentiations (IDs) family of nuclear oncogenes and their encoded proteins in glial tumours.

Results:

Structural analysis: The primary structure determined the composition of nucleotides and peptides. The target structure arranges by 468 nucleotides and 155 peptides with 56 peptides tied to DNA (**Table 1**). A three dimensional (3D) structure stated that the 56 polypeptides make a bHLH residue is a negative regulator recognized by two alpha-helix linked through a loop. The variability of the loop allows dimerization through folding and filling in the case of other helices. Those amphipathic alpha-helices have separated by a linker region of length (**Fig. 1a**). The Ramachandran diagram (ϕ , ψ plot) described the polypeptides locates in parallel and anti-parallel beta sheets (**Fig. 1b**).

Genome-wide analysis: The genome-wide analysis of both organisms by the HMMER algorithm obtained 72, 62 of bHLH domain in Homo sapiens and Mus musculus, respectively (**Table 2**). Standalone BLAST2 output represents 12, 13 homologs of inhibitor differentiation genes in Homo sapiens and Mus musculus, respectively (**Table 2**). The gene ontology annotation confirmed sequence accuracy of ID1-ID4 in the ID Family of bHLH TF's in Homo sapiens and Mus musculus (**Table 3 & 4**).

Domain, motifs, and phylogeny analysis: The highest hits of ID1 (target gene) listed from both organisms for sequence aligning, MSA results demonstrated conserved bHLH domain. The high consensus (90%) confirmed that the extended bHLH residue (**Fig. 2a**) and their specific motifs (**Fig. 3**). Further observation of the negatively regulated domain concluded that the ID1-ID4 conserved in evolution (**Fig. 2b**). The

experiment of the phylogenetic tree suggested the molecular evolutionary relationship of the ID Family of bHLH TF's in-between Homo sapiens and Mus musculus (**Fig. 4**).

Chromosome location, gene network, and expression analysis: Chromosome location study confirmed that the ID1 located band 20q11.21. Started 31,605,283 bp and, end 31,606,515 bp in humans (**Fig. 5**). The gene network study determined that the ID1 interacts with other molecules such as TCF3, TCF4, TCF12, RAP1A, ASCL3, THBS1, ETS2, ASCL1 also BMP2. Those molecular interactions govern the outcome of the ID1 gene in particular cells (**Fig. 6**). The disease state study in humans suggested the ID1-ID4 genes highly expressed in the neoplasm of the eye, brain, CNS, astrocytoma, glioblastoma, oligodendroglioma (**Fig. 7**) (**Table 5**). Therefore, the bHLH TF's data analysis concluded the total number of ID genes, peptide structure, conserved domain, motifs, phylogeny, chromosome location, gene network, and gene expression in isolated organisms.

Discussion:

The genomics study suggested the dominant outcome of the ID family of bHLH TF's revealed numerous hallmarks of development such as stem cell defence, cellular growth, differentiation, lineage determination, cell-cycle regulation, angiogenesis, vasculogenesis, migration, proliferation, tumorigenesis, immune response, and energy metabolism [1, 42, 51–58]. In the inhibitor differentiation (ID) family, ID1-ID4 share conserved domain and their sequence motif mediated dimerization through the sequestering of bHLH TF's such as E2A (TCF3), HEB (TCF12), and E2-2 (TCF4) are primarily the groups of E protein. The ID proteins have a negative DNA binding region (amino acids residues). But, ID proteins serve natural occurring dominant negative inhibitor of E proteins by the reaction of non-functional heterodimers. The ID1-ID4 has similar functions to suppress the DNA-binding activity of E proteins. The sequestering of E proteins suggested inhibitor differentiation proteins decrease reactions of heterodimers via tissue-specific bHLH polypeptides [59]. The stability of inhibitor differentiation proteins for the E proteins is complex and has discharge functions during sequestering by the motion of their structure. Hence, we can consider that the E proteins activity in the cells determines by the total concentration of E proteins subtracted by inhibitor differentiation proteins. The functional study supported inhibitor differentiation proteins engaged as an effective approach to delineate the collective activity of E proteins [60–62]. Precisely, the combination of inhibitor differentiation proteins, artificial molecule (recombination), and ET2 is supported and exploited. ET2 contains N-terminal polypeptides of E47 with two transcriptional residues and C-terminal polypeptides of SCL/TAL1 composed of the basic helix-loop-helix domain. Since the residues of SCL and TAL1 do not have to dimerize via ID proteins but has good stability for E protein [63]. But ET2 interact with E proteins greedily and bind to DNA sequences (E box) since ET2 contains transcriptional arouse domains of E47, which is heterodimers between ET2 and E proteins that raise transcription of target associated genes. Consequently, the ET2 compete with the ID family to coordinate the other proteins and neutralize the inhibitory impact of inhibitor differentiation proteins. Also, ID proteins resist the functions of E proteins through the interaction of various proteins without the bHLH domain. Such as ID2 associated with RB proteins that differentially repress G1/S and G2/M associated genes after P53 activations. That leads to an antagonistic relationship between ID2-RB [47, 40]. Indifference, the ID1 bind

to membrane-associated molecules regulates integrin signals (CAV1) [41, 64]. ID3 implicated to coimmunoprecipitate with the PAX5 protein and inhibit its transcriptional mobility [35]. Even the ID1/ID3 regulates in cell cycle process and transcribes the G1 phase by a reaction of serum stimulation. ID1 function promotes the outgrowth of NIH3T3 fibroblast during the adaption of the G1 to S phase. Besides, an elevated level of E47 suggested the E proteins arrest cell cycles through adaptation in NIH3T3 cells. These results are constant for E proteins implicated during transcriptional catalysts of the p16/p21 are inhibitors of the cyclin-dependent kinase. The link between inhibitor differentiation proteins and E proteins in cell cycle-regulated fashion suggested the E2A (E12 or E47) as homodimer initiate transcription of CDKs. So, antagonize ID proteins to E protein-initiated transcriptional catalysts of p16/p21 recognized as cell-cycle controllers. Other mechanisms suggested the resistance of ETS1 by inhibitor differentiation proteins controls the reaction of p16, a leading switch of the cyclin-D-dependent kinase [65–69]. Also, ID1/ID3 stimulates the response of genes complex in proliferation, invasion, and survival outside the E proteins [52]. In some circumstances, the ID1 attach to the p65 subunit of NF- κ B and enhance the NF- κ B targets genes. The formation of NF- κ B activity and the anti-apoptotic effector's genes is BCL-XL and ICAM-1 (CD54). Therefore, ID proteins can either function as pro-apoptotic or as anti-apoptotic molecules. ID1-transfected cells resistance by tumour necrosis factor (TNF) through the inactivation of BAX and CASPASE 3 [61, 70–72]. The ID1/ID3 in angiogenesis suggested function in the blood vessels of integrins (α 6, β 4, and α v β 3 integrins), FGFR1, and MMP2 by the response TSP-1. The above initiations are important for regulating bone-marrow-derived endothelial-cell attack and relocation. The recovery of angiogenesis impaired ID-deficient of HSP90 inhibitor, 17-allylamino-17-demethoxygeldanamycin or Tanespimycin suppresses HER2-neu-dependent manner [7, 74–76]. In fibroblasts, ID proteins promote the initiation of new blood vessels through the repression of TSP-1, a robust inhibitor during angiogenesis [74]. Additionally, ID proteins boost the function of VEGF. ID proteins prefer endothelial cells proficient for mobilization and proliferation of VEGF [10, 77, 78]. A shed light of BMP-dependent repression of ID1 through TGF β -specific SMAD2/SMAD3 requires synthesis via ATF (ATF3)/CREB family. The anomaly of ATF/CREB site for TGF β -initiated repression of promoter elements is fundamental for BMP signalling. Adhesion of ATF3 induces by the process of TGF β and cooperate naturally with TGF β -responsive SMAD3 but no BMP-specific SMAD1, enabling cells to distinguish between BMP and TGF β [79]. TGF β act as an inhibitor or activator of endothelial cells depends on two different TGF- β receptors: (a) ALK5 signalling via TGF β -sensitive SMAD2 and (b) ALK1, which produces and activates SMAD5 through BMP response. The aggregation of TGF β suggested ALK1 signalling via SMAD5 that accumulate migration and proliferation of endothelial cells by the function of ID1. Also, ALK5 suggested a high quantity of TGF β that inhibit endothelial cell proliferation and regeneration through induction of PAI [80]. Besides, TGF β and ID2 induce diverse cell lineages in the immune system. The trafficking of dendritic cells occupied by the TGF β directly initiates transcription of ID2. Precisely, early B-cell progenitors revealed TGF β 1 induced by the process of ID2/ID3. Also, ID3 adoption is prominent at the pro-and pre-B-cell stages, whereas ID2 initiation is powerful in mature B cells. Therefore, TGF β -mediated activity of ID2 function leads to IgE associated gene transcription and class switch recombination (CSR) [81–83]. In estimation, the ID2 function regulated by GFI-1 is zinc-finger proteins that act as a repressor. GFI-1 plays a dominant role in hematopoietic stem cells that maintenance even

binds to the ID2 promoter and inhibits transcription. Also, ID2 accord a preface in erythroid differentiation and promote the growth of erythroid lineage cells [84–86]. In a variation, the lipopolysaccharides (LPS) stimulate ID1 function in HSC. The effect of LPS potential attributes to transient functions of TNF α and IL-10 (inflammatory cytokines) increase turnover of HSC. These findings reveal the ID1 function initiate the HSC by process of LPS that promote TLR signalling [87]. Furthermore, the ID1 to an immunoglobulin enhancer component found at the 3'-end of gene negotiates transcriptional catalyst by responses of STAT5 and C/EBP β . ID1 function in myeloid tissue revealed CCAAT enhancer-binding proteins that play vital roles by cytokines such as IL-3 and GM-CSF activated STAT5. Another inflammatory cytokine of IL-6 also stimulates ID1 functions. Also, the ID2 function conveys to be initiated by C/EBP β . Invariance, ID3 inflicts RAS/MAPK initiation by responses of the EGR TF's [88–93, 63]. ID3 function in humoral immunity correlated with a low degree of IgG1 and IgG2 challenged the T-cell-dependent or T-cell-independent antigens that block thymocytes during the transition from single to double-positive cells. This functional mechanism suggested TCR (T-cell receptor) signalling enables ID3 to captivate several immune checkpoints during T cell maturation [7, 55, 94, 95]. In cancer biology, the ID family of bHLH TF's well characterized in diverse cancers such as glioblastoma, medulloblastoma, neuroblastoma, seminoma, prostate cancer, epithelial ovarian cancer, cervical cancer, endometrial cancer, breast carcinoma, melanoma, pancreatic carcinoma, head & neck cancer, medullary thyroid carcinoma, gastric cancer, T-cell lymphoma, B-cell leukaemia, colon carcinoma, and Ewing sarcoma [51–58]. ID genes function proposed as a prognostic signature in various cancers. In some conditions, it is adequate to render cells immortal or induce oncogenic mutation. Genomic stability of the ID family of bHLH TF's in molecular cancer therapy originates from the hypothesis that accumulates blocking of cellular differentiation and ability to drives proliferation. The ID family of bHLH TF's has negative functions to govern cellular differentiation and cell cycle regulation. Overwhelming evidence supported the resolution of ID genes act to enhance proliferative factors in different neural cell types. Also, the ID genes are a supreme regulator of proliferation in the NS. The functions of ID genes in neural growth suggested the encoded ID proteins control impulsive segregation and ultimately cell cycle block. These mobilities recognize by ID proteins to irritate bHLH TF's and tumour suppressor proteins (RB family). It is supported the ID1-ID4 proteins in post-natal tissues abnormally expressed in tumour endothelial cells attained from CNS and PNS [1]. During development, ID genes set the timing of differentiation in various neural cells includes neurons and oligodendrocytes. Dereglulation and malformed expressions of ID genes are associated with neo-angiogenesis, relentless proliferation, and lack of differentiation, a landmark of neural tumour progression [1]. ID2 play a key role in cell fate judgment and oncogenesis. The process of ID2 initiated the aliment of a neural crest [96]. ID2 function is increase by the robustness of N-MYC, a well-characterized regulator of segregation and proliferation in neural crest [41, 97]. ID2 activate by the enlightenment of chimeric proteins (N-MYC and EWS–ETS). The top degree of ID2 functions influence by the processes of EWS–ETS (fusion oncoproteins) and C-MYC. The targets of EWS–ETS are co-express with ID2/N-MYC that restrains the result of ID2 in the cellular process. Interestingly, ID2 function increase by the reaction of insulin growth factor (IGF) in pediatric neuroectodermal tumours [98–100]. Indifference, the NSCs revealed the self-renewal ability to originate all the major cells type in the NS. ID proteins maintain NSCs by regulating lineage commitment and prevent NSCs from premature differentiation. Precisely, ID2/ID4

blocks oligodendrocyte growth by inhibiting OLIG1/OLIG2 are bHLH TF's robust during oligodendrocyte growth [101]. Surprisingly, ID4 as a BRCA1-regulating gene and expression decreases BRCA1 and enhances tumorigenicity via HSP90 inhibitor in cancer cells. In addition, ID1-ID3 blocks premature differentiation by a function of HES1 that inhibits the expression of proneural genes. Also, the inhibitor differentiation proteins inhibit neuronal differentiation by binding with NeuroD and E47 complex to E-boxes. ID proteins emerge to sustain self-renewal ability in NSC for differentiation and stimulate proliferation. Notably, the p53 activity as a repressor of ID1/ID2 and p53 of NSCs raised ID functions and proliferation. This breakthrough is a vital phenomenon for treatments of cancer since p53 is necessary for a majority of glioblastoma [52, 76, 102–106]. Furthermore, ID1-ID4 proteins are illiberal with a short-life (<30 min) even the substrates of ubiquitin 26S proteasome system is a proteolytic molecule of eukaryotic cells [107, 108]. UB is an 8-kDa protein driven to ubiquitin-initiative enzyme E1 in ATP-dependent fashion and then to the ubiquitin-implicate enzyme E2. Generally, the ubiquitin covalently linked to the target protein by E3 ubiquitin ligase deploys to derive a polyubiquitin chain. The polyubiquitinated protein is rewarded by 26S proteasome and dehydrated in ATP dependent manner [53]. The E3 ubiquitin ligases are categorizing into four superior classes: (1) RING-finger-type, (2) U-box-type, (3) HECT-type, and (4) PHD-finger-type. The RING-finger-type subdivides into (a) Cullin E3 ligase and (b) Anaphasepromoting complex/cyclosome (APC/C). The E3 ubiquitin ligase of APC/C needed either CDC20 or CDH1 co-activators that bind the substrate via specific destruction box domains [53]. The ubiquitin/proteasome machinery includes two variable steps: (a) ubiquitination and (b) degradation. Ubiquitination mediated protein is tagged by abundant ubiquitin molecules recognized by proteasome complex from other proteins. Degradation of such multi-ubiquitinated proteins prevails on a massive 26S proteasome aggregation. Those mechanisms exposed that the cyclin-B synthesis is a regulated factor for the cells to drive mitosis. Even cyclin-B degradation is the central component that governs egress from mitosis and drives into the G1 phase of the next cell cycle. The cell cycle-dominated control of cyclin B-initiates catalyzes by ubiquitin/proteasome-dependent fashion. Similarly, cycling E synthesis controls the late G1 progression and breakdown of cycling by the ubiquitin/proteasome for cells to move in the S phase. Invariance, the CDK inhibitors (p21 and p27) are suppressors of p53 and pRB, and E2F-1 all degrade via ubiquitin/proteasome mechanism. In contrast, the accuracy negotiated by E3 ubiquitin ligase retains a high degree of specificity for the substrate [109–111]. The ubiquitin ligase is the pre-dominance of inhibitor differentiation proteins for proteasomal-initiated degradation through the cell-cycle regulator of APC/C. The APC/C and their co-activator of CDH1 (CD324) recognize ID1/ID2 and ID4 via conserved D-box motif situated C-terminus to the helix-loop-helix domain. Indeed, variations of the D-box of ID2 outcome accounted through a remarkable equilibrium of the substances. During the cellular process, APC6/CDC16, APC8/CDC23, and APC3/CDC27 are core components of APC/C are fundamental for the ubiquitination substrates. The ID1-ID4 proteins are essentially for targets of APC/C for the control of axonal growth in post-mitotic neurons via the signal of NOTCH1, Nogo receptor, SEMA3F, UNC5A, and JAG2 [3, 53, 112]. The degradation-resistant variation of ID2 acquired through mutations of a recognition site of APC/C (D-box) is sufficient to enhance axonal maturation and control inhibitory effects on axonal elongation imposed by myelin components. Besides, myelin of CNS inhibit neurite growth and stimulate the collapse of outgrowth cones through Nogo receptor, Nogo66, MAG, and OMPG molecules initiate

axon-repulsive signals by UNC5A and SEMA3F both participate in the regulation of myelination through the single of NOTCH and JAGGED. Therefore, ID1-ID4 proteins in post-mitotic neurons establish a novel loop among cancer and axonal regeneration. Also, dominant-negative antagonists prefer to induce cytoplasmic relocation of inhibitor differentiation proteins are the interferon-inducible protein p204. Interestingly, p204 promote the ubiquitin-initiated degradation of ID3 and probably remaining ID proteins activation required for ubiquitin ligase(s) [53, 113]. Therefore, the ubiquitin/proteasome executes a core function in the degradation of these regulatory proteins. Future work will require to achieve the targets in clinical cohorts. Ergo, the molecular functions even classical mechanisms epitomize the ID family of bHLH TF's is a novel regulator in tumour biology.

Materials And Methods:

Target Sequence and Database

The target sequence retrieves from the different specific databases (UniProt, KEGG, GenBank, EMBL, DDBJ and NCBI) and performs web-based application SMART for identification of the particular residue in the suspected sequence (query sequence). SWISS-MODEL performs for prediction of the protein structure is bioinformatics web-server for remodelling of the structure of molecules. This method is useful for generating molecular structure and utilizes it in many practical applications. The SWISS-MODEL is an updated database of remodelling of organism proteome for medical research.

Genome

Two organism's genome sequences downloaded from various exclusive databases (Ensemble and NCBI).

Standalone Tools

HMMER executes through MSA of the target domain as a profile search. HMMER is statistical algorithms that build by MSA of the suspected region for profile search. Is implemented probabilistic model is well-known as the profile Hidden Markov Model (HMM). Standalone BLAST2 executed for homologs gene in both organisms.

Gene Annotation

The BLAST2GO initialized for GO annotation. BLAST2GO is a computational and bioinformatics application for high-throughput GO annotation of particular sequences. The functional property of genes rectify via GO (Gene Ontology) annotation is a popular tool for practical work.

Domain

For observation of the conserved residue in the target sequence, we perform the MSA method to calculate unique tests of the homologs also streak them up, so we can observe the identity, differences and

similarities. MSA of highest hits sequences analysis conducted using web-based application MultAlin for examination of sustain domain.

Motifs

MEME suite application performs for the resolution of sequence motifs is a bioinformatics web-based tool for analysis and discovery of the specific motifs.

Phylogeny

For experimentation of the molecular evolutionary relationship of the particular gene in both organisms, we can perform MEGA-X for constructing a phylogenetic tree using Neighbor-Joining Methods.

Gene Expression

The gene expression analysis can carry out by GENEVESTIGATOR. GENEVESTIGATOR is an excessive-performance search engine for gene expression of different organisms. That application performs to determine and validate novel targets.

Chromosome Location

Chromosome location can retrieve using a web-based application that is well-known as a gene card. The gene card database provides information on all known and predicted genes. This database is currently available for biomedical research such as predictions of genes, encoded proteins and associated diseases.

Gene Networks

The genetic matrix (gene network) is a group of molecules that regulates and interact with one another in the cells to control the expression volume of mRNA or proteins. Many proteins serve to activate genes are the TF's that bind to the pioneer area and initiate the function of other proteins is called regulatory cascades. We can retrieve the STRING database for the prediction of protein-protein interaction. STRING database contains various resources like experimental data and computational prediction of proteins and nucleic acids.

Declarations:

Ethical approval:

Not applicable

Consent for Publication:

Not applicable

Availability of data and materials:

The data and materials are not deposits in the database.

Competing of interests:

The author declared that the work has no conflict of interest.

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Author Contributions:

The author proposed the idea, experimented, analyzed data and also prepared the manuscript.

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Tables:

Table 1: Target Sequence (Query Sequence)

>ID1

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atgaaagtcgccagtggcagcaccgccaccgccgccggggcccccagctgcgcgctgaag
gccggcaagacagcgagcggtgcggggcgaggtggtgcgctgtctgtctgagcagagcgtg
gccatctcgcgctgcgccggggggcgccggggcgcgccctgcctgccctgctggacgagcag
caggtaaactgctgctctacgacatgaacggctgttactcacgcctcaaggagctggtg
cccaccctgccccagaaccgcaaggtgagcaaggtggagattctccagcacgtcatcgac
tacatcagggaccttcagttggagctgaactcggaatccgaagttggaacccccgggggc
cgagggctgcgggtccgggctccgctcagcaccctcaacggcgagatcagcgccctgacg
gccgagggcgcatgcgttcctgcggacgatcgcattctgtgtcgctga
```

(a) Nucleotide

>ID1

```
mkvasgstataaagpscalkagktasgagevvrcclseqsvaisrcaggagarlpalld
eqqvnvlllydmngcysrlkelvptlpqnrvskveilqhvidyirdlqllelnsesevg
tpgggrglpvraplstlнгеisaltaeaacvpaddrilcr
```

(a) Peptide

Table 2: Summary of the bHLH domain and homologs

Organisms	HMMER	BLAST2	BLAST2GO
Homo sapiens	72	11	2
Mus musculus	62	13	2
Total	134	24	4

Table 3: Summary of the ID family of bHLH TF's

Gene	Homo sapiens	Mus musculus
ID1	2	2
ID2	3	3
ID3	2	1
ID4	1	1
Total	8	7

Table 4: Summary of the Gene Ontology annotation**(a) Homo sapiens**

Gene Id	Gene	Protein
ENSP00000365280.3	ID-1	DNA-binding inhibitor ID-1
ENSP00000365273.3	ID-1	DNA-binding inhibitor ID-1
ENSP00000379585.1	ID-2	DNA-binding inhibitor ID-2
ENSP00000385465.2	ID-2	DNA-binding inhibitor ID-2
ENSP00000234091.4	ID-2	DNA-binding inhibitor ID-2
ENSP00000489102.1	ID-3	DNA-binding inhibitor ID-3
ENSP00000363689.5	ID-3	DNA-binding inhibitor ID-3
ENSP00000367972.3	ID-4	DNA-binding inhibitor ID-4

(b) Mus musculus

Gene Id	Gene	Protein
ENSMUSP00000092019.4	ID-1	DNA-binding protein inhibitor ID-1
ENSMUSP00000105449.1	ID-1	DNA-binding protein inhibitor ID-1
ENSMUSP00000020974.6	ID-2	DNA-binding protein inhibitor ID-2
ENSMUSP00000152052.1	ID-2	DNA-binding protein inhibitor ID-2
ENSMUSP00000152069.1	ID-2	DNA-binding protein inhibitor ID-2
ENSMUSP00000008016.2	ID-3	DNA-binding protein inhibitor ID-3
ENSMUSP00000021810.1	ID-4	DNA-binding protein inhibitor ID-4

Table 5: ID family of bHLH TF's in Primary Human Tumors

Gene	Tumor Type	Reference
ID1, ID3	Glioblastoma	Lyden et al., 1999
ID1, ID3	Medulloblastoma	Lyden et al., 1999
ID1, ID3	Neuroblastoma	Lyden et al., 1999
ID1, ID2, ID3	Astrocytic tumor	Vandeputte, D.A. et al., 2002
ID1, ID2, ID3	Pancreatic cancer	Maruyama et al., 1999
ID1, ID2, ID3	Head and Neck cancer	Langlands, K. et al., 2000
ID1, ID2, ID3	Colorectal adenocarcinoma	Wilson, J.W. et al., 2001
ID1, ID2, ID3, ID4	Seminoma	Sablitzky et al., 1998
ID1, ID2	Pancreatic cancer	Maruyama, H. et al., 1999
ID1, ID2	Pancreatic cancer	Lee, K.T. et al., 2004
ID1, ID2	T-cell lymphoma	Kim, D. et al., 1999
ID1, ID2	T-cell lymphoma	Morrow, M.A. et al., 1999
ID1	Medullary thyroid cancer	Kebebew et al., 2000
ID1, ID2, ID3	Squamous cell cancer	Langlands et al., 2000
ID1	Breast cancer	Lin et al., 2000
ID1	Breast cancer	Fong, S. et al., 2003
ID1	Breast cancer	Schoppmann, S.F. et al., 2003
ID2	Breast cancer	Itahana, Y. et al., 2003
ID3	Breast cancer	de Candia, P. et al., 2003
ID4	Breast cancer	Beger et al., 2001
ID1	Endometrial cancer	Takai et al., 2001
ID1	Cervical cancer	Schindl et al., 2001
ID1	Melanoma	Polsky et al., 2001
ID2	Neuroblastoma	Lasorella et al., 2002
ID2	Ewing's sarcoma	Fukuma, M. et al., 2003
ID2	Ewing's sarcoma	Nishimori, H. et al., 2002
ID1	Ovarian tumors	Schindl, M. et al., 2003
ID3	Ovarian tumors	Arnold, J.M. et al., 2001

ID1	Prostate cancer	Ouyang, X.S. et al., 2002
ID1	Prostate cancer	Coppe, J.P. et al., 2004
ID1	Esophageal cancer	Maruyama, H. et al., 1999
ID1	Oral cancer	Nishimine, M. et al., 2003
ID1	Melanoma	Polsky, D. et al., 2001
ID1	Hepatocellular cancer	Lee, T.K. et al., 2003
ID4	Acute lymphoblastic leukemia	Bellido, M. et al., 2003

Figures

(a)

(b)

Figure 1

(a) Tertiary Structure of ID1 (Target/Query Sequence) (b) ID1 polypeptides position in Ramachandran Plot (Ramachandran Diagram)

1 10 20 30 40 50 60 70 80 90 100 110 120 130

ENSP00000365280.3 MKVRSGSTATAAGPSCALKAGKTASGAGEVVRCLSEQSVATSRGAGAGARLPALLDEQQVNVLLYDHNGCYSRLKELVPTLPQNRKYSKVEILQHVIDYITROLQLELNSESEVGTTPGGRGLPVRAPLS
 ENSP00000365273.3 MKVRSGSTATAAGPSCALKAGKTASGAGEVVRCLSEQSVATSRGAGAGARLPALLDEQQVNVLLYDHNGCYSRLKELVPTLPQNRKYSKVEILQHVIDYITROLQLELNSESEVGTTPGGRGLPVRAPLS
 ENSHUSP00000092019.4 MKVRSGSAA-AAAGPSCSLKAGRTA---GEVVLGLSEQSVATSRGAG---TRLPALDEQQVNVLLYDHNGCYSRLKELVPTLPQNRKYSKVEILQHVIDYITROLQLELNSESEVGTTPGGRGLPVRAPLS
 ENSHUSP00000105449.1 MKVRSGSAA-AAAGPSCSLKAGRTA---GEVVLGLSEQSVATSRGAG---TRLPALDEQQVNVLLYDHNGCYSRLKELVPTLPQNRKYSKVEILQHVIDYITROLQLELNSESEVGTTPGGRGLPVRAPLS
 Consensus MKVRSGSAA-AAAGPSCSLKAGRTA...GEVVLGLSEQSVATSRGAG...TRLPALDEQQVNVLLYDHNGCYSRLKELVPTLPQNRKYSKVEILQHVIDYITROLQLELNSESEVGTTPGGRGLPVRAPLS

131 140 150 160 170 175

ENSP00000365280.3 TLNGEISALTAEEACVPPADDRILCR
 ENSP00000365273.3 TLNGEISALTAEEVRSRSDH
 ENSHUSP00000092019.4 TLNGEISALTAEEACVPPADDRILCR
 ENSHUSP00000105449.1 TLNGEISALTAEEVRSRSEAYYIILQMETEATGGGCPPSLFFRIARI
 Consensus TLNGEISALTAEEACVPPADDRILCR.....

(a)

1 10 20 30 40 50 60 70 80 90 100 110 120 130

ENSP00000365280.3 MKVRSGSTATAAGPSCALKAGKTASGAGEVVRCLSEQSVATSRGAGAGARLPALLDEQQVNVLLYDHNGCYSRLKELVPTLPQNRKYSKVEILQHVIDYITROLQLELNSESEVGTTPGGRGLPVRAPLS
 ENSHUSP00000092019.4 MKVRSGSAA-AAAGPSCSLKAGRTA---GEVVLGLSEQSVATSRGAG---TRLPALDEQQVNVLLYDHNGCYSRLKELVPTLPQNRKYSKVEILQHVIDYITROLQLELNSESEVGTTPGGRGLPVRAPLS
 ENSP00000367972.3 MKAVSPVPSGRKAPSGCGGGELALRCLAEHGHSLGGSAARAAAAAARCKAREAAADEPAL-CLQCDDHNCYSRLRLVPTIPPHKKYSKVEILQHVIDYITROLQLELNSESEVGTTPGGRGLPVRAPLS
 MKAVSPVPSGRKAPSGCGGGELALRCLAEHGHSLGGSAARAAAAAARCKAREAAADEPAL-CLQCDDHNCYSRLRLVPTIPPHKKYSKVEILQHVIDYITROLQLELNSESEVGTTPGGRGLPVRAPLS
 ENSP00000379585.1 MKAFSPVRSVRKNSLSDHSLGTSRSKTPVDOPH---SLLYNHNCYSRLKELVPSIPQNKYSKVEILQHVIDYITROLQLELNSESEVGTTPGGRGLPVRAPLS
 ENSHUSP00000020974.6 MKAFSPVRSVRKNSLSDHSLGTSRSKTPVDOPH---SLLYNHNCYSRLKELVPSIPQNKYSKVEILQHVIDYITROLQLELNSESEVGTTPGGRGLPVRAPLS
 ENSP00000489102.1 MKALSPVRGCEYAVCCLSERSLATARGRGKSPAEPL-SLLDHHNCYSRLRELVPGVPRGTQLSQVEILQVVIDYITROLQVVL-AEPAGPPDPGPHLPITQTAEL
 ENSHUSP0000008016.2 MKALSPVRGCEYAVCCLSERSLATARGRGKSPAEPL-SLLDHHNCYSRLRELVPGVPRGTQLSQVEILQVVIDYITROLQVVL-AEPAGPPDPGPHLPITQTAEL
 ConsensusL..V.....V...S..R..A.....R..K..P..D...L..S..L..H..H..CYSRLRELVP..P..n..kyskVEILQHVIDYITROLQ..L..epa.g....gp..IP..q....

131 140 150 160 165

ENSP00000365280.3 TL-----NGEISALTAEEA---CV--PADDRILCR
 ENSHUSP00000092019.4 TL-----NGEISALTAEEA---CV--PADDRILCR
 ENSP00000367972.3 TCPAPPPRTPLTALNTDPA---GAVNKGQDSILCR
 ENSHUSP00000021810.1 ACPVAPPRTPLTALNTDPA---GAVNKGQDSILCR
 ENSP00000379585.1 RTPLTTLNTDISILSLQASEFPSELNSHNSKALCG
 ENSHUSP00000020974.6 RTPLTTLNTDISILSLQASEFPSELNSHNSKALCG
 ENSP00000489102.1 IPELVISDKRSFCH
 ENSHUSP0000008016.2 IPELVISDKRSFCH
 Consensus L..L...n...s..l.....Lc.

(b)

Figure 2

(a) ID1 conserved in both organisms (b) ID1-ID4 conserved in two organisms (Multiple Sequence Alignment)

(a)

(b)

(c)

Figure 3

Sequence motifs of ID1 (Motifs)

Figure 4

The evolutionary link between the ID family of bHLH TF's in two different organisms. (Phylogenetic Tree)

Figure 5

(b) ID1 expression in Human Brain, (d) ID2 expression in Human Brain, (f) ID3 expression in Human Brain, (h) ID4 expression in Human Brain (Gene Expression of ID1-ID4 in Human)

Figure 6

ID1 in humans located at chromosome 20 (Chromosome location)

Figure 7

ID1 interact with various TF's (Gene network)