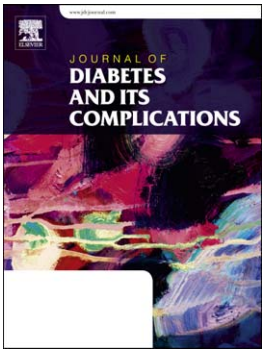

Vitreous biomarkers in diabetic retinopathy: A systematic review and meta-analysis.

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Vitreous biomarkers in diabetic retinopathy: A systematic review and meta-analysis.

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ABSTRACT

The aim of this study was to perform a systematic meta-analysis of biomarkers investigated with diabetic retinopathy (DR) in the vitreous, and to explore the molecular pathway interactions of these markers found to be consistently associated with DR. Relevant databases [PubMed and ISI web of science] were searched for all published articles investigating molecular biomarkers of the vitreous associated with DR. Based on set exclusion/inclusion criteria available data from studies with human vitreous samples were extracted and used for our meta-analysis. The interactions of significant biomarkers in DR were investigated via STRING and KEGG pathway analysis. Our meta-analysis of DR identifies eleven biomarkers as potential therapeutic candidates alternate to current anti-VEGF therapy. Four of these are deemed viable therapeutic targets for PDR; ET receptors (ET A and ET B), anti-PDGF-BB, blocking TGF-β using cell therapy and PEDF. The identification of supplementary or synergistic therapeutic candidates to anti VEGF in the treatment of DR may aid in the development of future treatment trials.

INDRODUCTION

Diabetic retinopathy (DR) is a common complication of both type 1 (T1DM) and type 2 (T2DM) diabetes mellitus (DM) and threatens the vision of an estimated 101 million people worldwide [1, 2]. Approximately one third of adults in the United States of America with DM have DR and a worldwide prevalence of 155 million is expected by 2030 [1, 3]. Vision loss from DR can occur due to either proliferative DR (PDR) or diabetic macula edema (DME), which both often manifest together, suggesting some commonality in causative and pathogenetic factors. However, while the importance of optimal glycemic and hypertension control in the prevention of DR and DME progression is well documented, clinically it is difficult to predict the clinical course of DR in some patients based on published risk factors [4-6]. This has encouraged the scientific study of local metabolic factors in the eye in an attempt to better understand the pathogenesis of DR.

Regarding vitreous dynamics and retinal interplay, it has long been recognized that vascular endothelial growth factor (VEGF) plays a crucial role in diabetic eye disease pathogenesis [7]. VEGF has been shown to stimulate neovascularisation and vascular permeability, causative in both PDR and DME. Following identification of VEGF both intraocular and systemically, anti-VEGF therapy has been increasingly utilized to inhibit retinal angiogenesis in PDR, and stabilize leakage in DME in affected cohorts. However, although anti-VEGF is effective for DR treatment and represents a paradigm shift in effective therapy, adverse effects have been noted namely; the slight but identifiable increased risk of tractional retinal detachment in vulnerable PDR cases, and thromboembolic events from systemic uptake [8-12]. As VEGF has the ability to cross the blood-retinal barrier it could be detrimental on the vascular and neural tissue [13, 14]. Additional limitations include the short effective duration of therapy

necessitating frequent administration, and those subsets of patients that fail to effectively respond to anti-VEGF treatments and are thus refractory to therapy [8, 12, 13]. Therefore, newer alternate therapies for DR would provide additional therapeutic agents in the treatment of DR.

Additional biomarkers associated with DR may allow synergistic pharmacological blockade or enhancement of biomarker activity improving the clinical phenotype. Identification of biomarkers in the vitreous, particularly in relation to PDR may allow an assessment of ocular specific therapy for vision loss in PDR. Identification of such biomarkers may thus aid in early detection, risk prediction in high risk patients, future treatment options and increase our understanding of disease pathogenesis, bridging the gap between epidemiological studies and risk factor prediction for progression.

Herein, we report on a systematic review and meta-analysis of molecular biomarkers for DR found in the vitreous. The aim of this study was to identify markers which are consistently associated with DR throughout various populations and conditions; define their molecular interactions; and further explore relevant pathological pathways and alternate therapies for DR.

MATERIALS AND METHODS

Search strategy and eligibility criteria This systematic review was performed in accordance with the PRISMA guidelines [15]. Published articles were extracted from the following databases: PubMed (NL) (http://www.ncbi.nlm.nih.gov/*pubmed)*; science citation index at web of science (ISI) (http://apps.isiknowledge.com); web of science (ISI) (http://apps.isiknowledge.com); Cochrane Library (http://acc.cochrane.org/cochrane-library). The following search terms: “biomarker”; “antibody”; “protein”; “histone”; “epigenetic”, “RNA”, were searched independently with the terms “vitreous”, “diabetic retinopathy” and “human” and repeated using

the term "patient". Search dates were left open (1963 until Sep 2012) to include all available research. All articles were retrieved and then searched for duplicates, firstly using Endnote's Endnote X3, (Thomson Reuters, Carlsbad, CA, USA). Subsequent manual checking of title, year of publication and author was conducted.

Articles were grouped based on title and abstract screening. Inclusion criteria were defined as; research presenting results from non-genetic, human, vitreous samples (Figure I). Exclusion criteria included; in vitro/in vivo studies, review articles, diabetic studies with no reference to DR, interventional clinical trials with pharmacological agents, and DR as mentioned in other non-diabetic diseases.

Data Extraction Data from all eligible studies were entered into a database and grouped according to author, year, biomarker, the severity of DR and type of DM. Following data entry, one in five papers were randomly selected for an independent checking of extraction errors prior to analysis. Recorded characteristics included study demographic features; sample size, age range, DM duration, DM subtype and HbA1c levels. For each investigated molecular biomarker, the mean, standard deviation (SD), range, confidence interval (CI) and reported significant level *p*-values was recorded for each study group reported, where applicable. A completed dataset was defined as a p-value and or a mean, standard and deviation with the unit of measure. Given the change in molecular nomenclature with time, and different abbreviations of the same biomarker across articles, all biomarkers studies were assessed for consistency using the UniProt (http://www.uniprot.org/) and NCBI (http://www.ncbi.nlm.nih.gov/) databases. Markers with three or more datasets per sub-group were included in the meta-analysis.

Statistical analysis Data were sub-grouped into biomarkers based on their unique UniProt or NCBI number. Within each biomarker subgroup the units of measure were standardized. Unit conversions occurred to ensure homogeneity of units between studies for each biomarker. For each biomarker, data were categorized by type of DM (T1DM and T2DM) and severity of DR (none; DME, DR severity unspecified, non-PDR (NPDR); and PDR). Comparisons were performed for studies stratified by DM subtype (T1DM and T2DM) and by study control group. These control groups were classified as being either diabetic patients without retinopathy or non-diabetic patients without retinopathy undergoing vitreoretinal surgery (macular hole, epiretinal membrane or retinal detachment).

Where possible mean fold change (MFC) between case and control groups was calculated from the weighted means and standard deviation, and forest and funnel plots were constructed in the Cochrane program Revman (http://ims.cochrane.org/revman). The Stouffers's method was run using R statistical package (http://www.r-project.org/) for the remaining analysis, which uses weighted z scores from the p-values of each individual study analysis [16]. A p value <0.05 was considered statistically significant in all analyses.

Pathway analysis Using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) version 9.0 (http://string-db.org*)*, a database which categorizes direct (physical) and indirect (functional) associations for known and predicted gene and protein expression interactions. Interactions are derived from four source types; genomic context, high-throughput experiments, conserved co-expression and previous published knowledge. To explore potential biological functional pathways with clusters of biomarkers a Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was run using GeneTrail (http://genetrail.com). The

identified proteins significantly associated with PDR (P15692; P05231; P10145; P14210; P01137; P01375; P36955; P05305; P01588; A0PJJ7; P01127; Q6UZ82) were run against the allGenes reference set, using a false discovery rate adjustment with a p-value < 0.05.

RESULTS

Systematic review and quantitative assessment After the exclusion of duplicate references, a total of 870 studies were identified. The search yield and primary reasons for exclusion are presented in Figure 1. On review of the abstracts, 222 clinical trials, 68 reviews, 88 in vitro/in vivo studies were excluded. Of the remaining, 84 studies were published in languages other than English, 15 were solely genetic and 67 had vitreous data previously reported, and were therefore excluded. After full text review, a further 11 studies had major contrary variables, such as drug delivery trials, compromising their generalizability and were therefore excluded. A total of 293 studies were found to meet our full inclusion criteria. Therefore, from 293 articles that underwent full test assessment 118 articles with 389 complete datasets could be included. There were 154 biomarkers identified in association with DR, and of these 15 had been reported from more than three independent samples from separate studies. A total of 12 biomarkers were assessable in relation to PDR in T1DM & T2DM combined, one biomarker in PDR T2DM, one in NPDR in T2DM; compared with non-diabetic mellitus patients who underwent vitrectomy (non-DM vitrectomy control); macular hole, epiretinal membrane or retinal detachment. For DME, one biomarker was assessable in T2DM compared to diabetics with and without retinopathy controls. A summary of the characteristics of all studies included is displayed in online supplemental data Table 1.

Meta-analysis results relative to all non-DM vitrectomy controls for 15 datasets assessable in the PDR, NPDR and DME categories are presented in Table 1. Ten biomarkers were significantly increased in the vitreous of those with PDR in T1DM and T2DM combined, when compared to non-DM vitrectomy control groups; VEGF, interleukin-6 (IL-6), interleukin-8 (IL-8), erythropoietin (EPO), platelet-derived growth factor BB chain (PDGF-BB), nitric oxide (NO), endothelin-1 (ET-1), monocyte chemotactic protein 1 (MCP-1), transforming growth factor beta (TGF-β), and tumor necrosis factor alpha (TNF-α). Pigment epithelium derived factor (PEDF) and hepatocyte growth factor (HGF) were significantly decreased in the vitreous of those with PDR in T1DM and T2DM combined, when compared to non-DM vitrectomy controls. Similarly, PEDF was decreased in the vitreous of those with DME in T1DM and T2DM combined when compared to non-DM vitrectomy controls. VEGF was also significantly increased in the vitreous of T2DM with PDR, and not significantly increased in the vitreous of those with NPDR in T1DM and T2DM combined, when compared to non-DM vitrectomy controls.

Molecular pathway analysis The STRING pathway analyses for the 12 biomarkers significantly associated with T1DM and T2DM in PDR are illustrated in *Figure 2*. STRING pathway analysis revealed VEGF could be activated by IL-6, IL-8, HGF, EPO, TNF-α, ET-1, and inhibited by HGF and ET-1. IL-8 can be activated by VEGF, TNF-α, HGF and ET-1. IL-6 can be activated by ET-1, TNF-α, MCP-1, and inhibited by HGF. MCP-1 can be activated by IL-6, IL-8 and inhibited by TNF-α. PEDF can be activated by VEGF. TNF-α can be inhibited by EPO. Interaction effects were found between a number of proteins (in particular: PDGFBB with HGF;

PDGFBB with IL-6; ET-1 with NO; TNF-α with HGF; IL-8 with Il-6; TGF-β with IL-6; TNF-α with ET-1; and IL-6 with EPO).

The KEGG pathway analyses for the 12 biomarkers significantly associated with T1DM and T2DM in PDR is presented in online supplemental data Table 2. Analyses revealed that the cytokine-cytokine receptor interaction pathway was significantly involved (p=3.16 x 10^{-10}) in association with 9 of the PDR associated proteins; VEGFA, IL-6, IL-8, HGF, TNF-α, TGF-β, EPO, PDGFBB, MCP-1. Further, it highlighted that TNF-α is also involved in the T1DM pathway.

DISCUSSION

An assessment of biomarkers in the vitreous associated with PDR, NPDR and DME has allowed for the identification of robust candidates for ocular specific markers of vision loss in patients with DR. Our meta-analysis supports the strong association between PDR and VEGF-A in the vitreous, but showed there was no statistically significant association with the less severe phenotype (NPDR). Various therapies have been proposed for modulating high levels of intraocular VEGF in PDR [17, 18]. Although, intravitreal anti-VEGF is currently being used in PDR treatment, anti-VEGF delivered intravenously has also shown some efficacy in advanced stages of DR [17, 19]. Therapeutic targeting of VEGF receptors, to modulate response, have proved effective in a mouse model of retinal ischemia, suggesting gene therapy may have an important role in the treatment of PDR in the future [20]. Further, our results reveal there are eleven statistically significant biomarkers for PDR and one for DME, apart from VEGF, as potential therapeutic candidates for these two conditions. We will discuss each of these candidates and provide information on their interaction with VEGF.

The molecular pathway analysis illustrated VEGF-A can be activated by five biomarkers that we found to be consistently significantly associated with PDR in the vitreous namely; IL-6, IL-8, EPO, TNF-α, HGF. One biomarker, MCP-1, interacts indirectly with VEGF-A via IL8 and TNF-α. IL-8, TNF-α and MCP-1 are important mediators of the innate immune response system [21, 22]. Multifunctional cytokines IL-6 and HGF act dually as pro-inflammatory mediators in response to tissue damage, and as anti-inflammatory mediators by initiating the suppression of adaptive immunity [23]. In chronic inflammatory conditions like DM, cell homeostasis is affected, with over-activation of innate immunity leading to inflammation, and providing protection via immune response suppression. Treatment options could involve general anti-inflammatory drugs or endothelium specific cannabinoid therapy that targets multiple cytokines [24, 25]. While these inflammatory biomarkers are not explicitly associated with DM, they could be utilized as specific ocular therapeutic targets in combination with VEGF to mediate the immune response in PDR.

Currently EPO is hypothesized as a novel treatment for early DR, due to its neural protective effect on the diabetic retina in-vivo [26]. Our meta-analysis also revealed an increase in EPO in the vitreous at the PDR stage also, possibly a reflection of vitrectomy requiring sampling bias, however, highlighting that EPO is also present in later severe stages of DR. A clinical feature of PDR, ischemia, stimulates the glycoprotein EPO which in turn activates VEGF, demonstrating EPO is precursory to VEGF, as illustrated in our STRING pathway analysis [27, 28]. Additionally, EPO demonstrates stimulatory angiogenic activity in the microvasculature and red cell proliferation while preventing cell apoptosis, all actions contributory to neovascularization in PDR [27-29]. Future studies will need to investigate EPO

optimization for early DR treatment, due its actions on VEGF and contribution to neovascularization in PDR.

ET-1 is an intriguing candidate as it is both inhibited by VEGF-A, and yet still over expressed in the vitreous of those with high levels of VEGF-A from PDR. In-vitro studies suggest activation is most likely due to hyperglycemia and or insulin, and an increase in ET-1 reflects a prominent manifestation of the diabetic state [30, 31]. ET-1 is a vasoconstrictor which also stimulates cell proliferation [32], thus could be considered a potential therapeutic candidate for PDR. However, it is the endothelial receptors (ET A and ET B) that are hypothesized to be protective for vascular structure [33, 34], suggesting targeting the receptor could be useful in PDR therapy. As recently recommended, future studies will need to prove selectivity for ET-1 in the microvasculature before receptor antagonists can be utilized for PDR treatment [35].

NO has been shown by our meta-analysis to be significantly increased in the vitreous of patients with PDR. NO, similar to ET-1, is activated by hyperglycemia and influences blood flow within the microvasculature by functioning as a vasodilator [36, 37]. Low levels of NO are key to maintaining cell homeostasis, by regulating cell proliferation and survival [36, 38]. However, over expression of NO can be detrimental to retinal and endothelial cells leading to increased cell death and impaired proliferation [39, 40]. Recently, the NO donor drug has been trialed in endothelial progenitor cells from patients with T2DM to reverse the effects of high glucose on cell proliferation deregulation [41]. However, it has been suggested that for this to be an effective treatment of PDR, future studies would need to address how NO can be selectively reduced intraocularly without damaging the protective role of NO levels in the retina and endothelial cells [42].

We reveal that the PDR biomarker candidate PDGF-BB is consistently increased in the vitreous of patients with PDR. PDGF-BB has been attributed to be causal in neovascularization, a clinical feature of PDR [43]. This is supported by recent in vivo/in vitro trials and an initial clinical trial demonstrated the synergistic combination of anti-VEGF & anti-PDGF-BB reduced PDR response more than anti-VEGF alone [43, 44], suggesting anti-PDGF-BB could be used synergistically with anti-VEGF in future therapeutic treatment of PDR.

We found increased levels of TNF-α in the vitreous of those with PDR, however, as reflected by our KEGG pathway analysis, this polypeptide is up regulated in T1DM. An increase in TNF-α is most likely a reflection of the diabetic state in T1DM. TNF-α is an early inflammatory marker in response to hyperglycemia that has been suggested to be contributory to the clinical features of PDR; angiogenesis and neovascularization [45, 46]. Therefore, targeting TNF-α therapeutically could be beneficial for PDR. In a diabetic in vivo model TNF-α was shown to decrease in the retina in response to non-nucleoside adenosine kinase inhibitor that shows protective therapeutic value for DR, however it has yet to be systematically tested in a PDR model [47].

TGF-β is considered a likely therapeutic target for PDR, and our meta-analysis showed considerably increased levels within the vitreous of those people with PDR [48]. TGF-β is a potent mediator of the inflammatory and immune response in the retina by mediating the blood retinal barrier and endothelial permeability. At adequate levels TGF-β is required to maintain microvasculature homeostasis [49-51]. Suggesting increased levels of TGF-β may play an important role in the breakdown of the blood retinal barrier and microvasculature dysfunction, as seen in PDR. Recent tests using stem cells from diabetic patients show that blocking TGF-β

enhances vessel repair, therefore, future potential PDR treatment may involve cell therapy [48, 52].

Our meta-analysis revealed PEDF is significantly decreased in the vitreous of patients with both DME and PDR. PEDF is part of the serine protease inhibitor superfamily, which has been found to suppress neovascularization, a clinical feature of PDR [53]. Recently PEDF was suggested as a potential therapeutic inhibitor of vascular neovascularization in DR [54, 55]. In vivo models have shown intravitreal injection of PEDF reduces retinal neovascularization [56, 57]. Suggesting PEDF would be a good therapeutic candidate for PDR for its suppression of neovascularisation and possible benefits in relieving edema [53].

These exciting potential candidates for DR were found, along with VEGF to be involved in the cytokine-cytokine receptor interaction pathway via our KEGG analysis. This pathway aids to regulate angiogenesis, inflammation and immunosuppression, thus complementing the known PDR clinical features of inflammation; vasodilation, changes in microvasculature, leukocyte migration and edema [21-23]. The significant involvement of inflammatory cytokines and growth factors in the vitreous as documented across multiple studies adds further weight to the role of inflammation in PDR.

Limitations of this study include the inability to assess T1DM and T2DM separately as the majority of studies combined the DM subtypes. Indeed, future studies should consider separating T1DM and T2DM cohorts to ascertain biomarker differences between the DM subtypes. Further limitations arose from the non-DM vitrectomy controls; macular hole, epiretinal membrane or retinal detachment, utilized by studies, due to difficulty in vitreous sample acquisition from ocular healthy persons. Therefore, markers could reflect inflammation from DM and not be specific for the PDR, NPDR or DME phenotypes. Specifically, our KEGG

pathway analysis identified that TNF-α is considered a marker of T1DM. Future studies could try to sample vitreous from diabetic controls. Anterior chamber sampling may also provide additional sampling potential for the early diabetic phenotypes in a less invasive manner. Nevertheless, results should not be dismissed as VEGF was identified with non-DM vitrectomy controls [7]. Indicating biomarkers elevated in the vitreous from DM and/or DR can provide insight into the functioning of the vitreous of those with diabetes and provide potential avenues for the clinical treatment of DR.

Our previous meta-analysis on blood derived biomarkers in DR compared to diabetics without retinopathy found that significant biomarkers in the VEGF pathway can be detected in the plasma of those with PDR; ICAM-1 & VCAM-1 [58]. Of particular interest was our previous meta-analysis showed VEGF is at minor levels in the peripheral blood in PDR [58], indicating, the VEGF response in PDR may be primarily ocular specific, but the VEGF pathway markers are detectable systemically. It also appears cytokines present in the vitreous are not directly mirrored in the vascular system [22, 59].

In conclusion, the imperative search for additional biomarkers involved in the clinical manifestations of DR has revealed 11 biomarkers alternative to VEGF in the vitreous of patients with PDR. All should be considered as potential therapeutic candidates for PDR, however, four are currently viable therapeutic candidates that could be trialed alternatively to current anti-VEGF therapy for PDR; endothelial receptors (ET A and ET B), anti-PDGF-BB, blocking TGF-β using cell therapy and PEDF. Potentially the most effective therapies may utilize multiple candidates that work synergistically to alleviate the symptoms of PDR. Finally, the method of identifying candidate markers coupled with pathway analysis revealed multiple molecules

involved in the underlying pathophysiological mechanisms in DR, and has progressed our understanding of the molecular interactions in the vitreous in the pathogenesis of DR.

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Table 1. Vitreous biomarkers in association with PDR, NPDR and DME.

Biomarker	DM type	DR type	Control type	Number of studies	Number of cases	Number of controls	Direction of change	p-value
VEGF	T1DM & T2DM	PDR	Non diabetic‡	33	940	669	Increased in PDR	1.16×10^{-51}
IL-8	T1DM & T2DM	PDR	Non diabetic‡	4	150	68	Increased in PDR	8.58×10^{-12}
IL-6	T1DM & T2DM	PDR	Non diabetic‡	6	291	147	Increased in PDR	1.73×10^{-9}
HGF	T1DM & T2DM	PDR	Non diabetic‡	5	149	65	Decreased in PDR	1.11×10^{-09}
EPO	T1DM & T2DM	PDR	Non diabetic‡	3	144	99	Increased in PDR	1.89×10^{-6}
PDGF-BB	T1DM & T2DM	PDR	Non diabetic#	4	147	63	Increased in PDR	1.48×10^{-5}
NO	T1DM & T2DM	PDR	Non diabetic‡	3	40	32	Increased in PDR	1.00×10^{-5}
PEDF	T1DM & T2DM	PDR	Non diabetic‡	3	77	51	Decreased in PDR	1.00×10^{-5}
ET-1	T1DM & T2DM	PDR	Non diabetic‡	3	42	40	Increased in PDR	1.00×10^{-5}
MCP-1	T1DM & T2DM	PDR	Non diabetic‡	3	101	48	Increased in PDR	2.00×10^{-5}
TGF-β	T1DM & T2DM	PDR	Non diabetic†	3	69	37	Increased in PDR	0.002
TNF-α	T1DM & T2DM	PDR	Non diabetic‡	4	156	73	Increased in PDR	0.03
VEGF	T2DM	PDR	Non diabetic‡	3	129	82	Increased in PDR	3.47×10^{-9}
VEGF	T1DM & T2DM	NPDR	Non diabetic‡	3	52	46	Increased in NPDR	0.65
PEDF	T1DM & T2DM	DME	Non diabetic‡	3	103	32	Decreased in DME	1.13×10^{-9}

‡ Non diabetic mellitus control type defined as studies that included non diabetic mellitus patients whom underwent vitrectomy; macular hole, epiretinal membrane or retinal detachment.

Non diabetic; MH, ERM, retinopathy
† Non diabetic; vitrectomy (ERM, MH) and cadaver

Abbreviations: T1DM: type 1 diabetes mellitus, T2DM: type 2 diabetes mellitus, VEGF: vascular endothelial growth factor; IL-8: interleukin-8, IL-6: interleukin-6; HGF: Hepatocyte growth factor, EPO: erythropoietin, PDGF-BB: platelet-derived growth factor BB chain, NO: nitric oxide, PEDF: pigment epithelium-derived factor, ET-1: endothelin-1, MCP: Monocyte chemotactic protein 1, TGF-β: transforming growth factor beta 1, TNF-α: tumor necrosis factor-alpha.

Figure 1. Flow Diagram of Study Selection Process

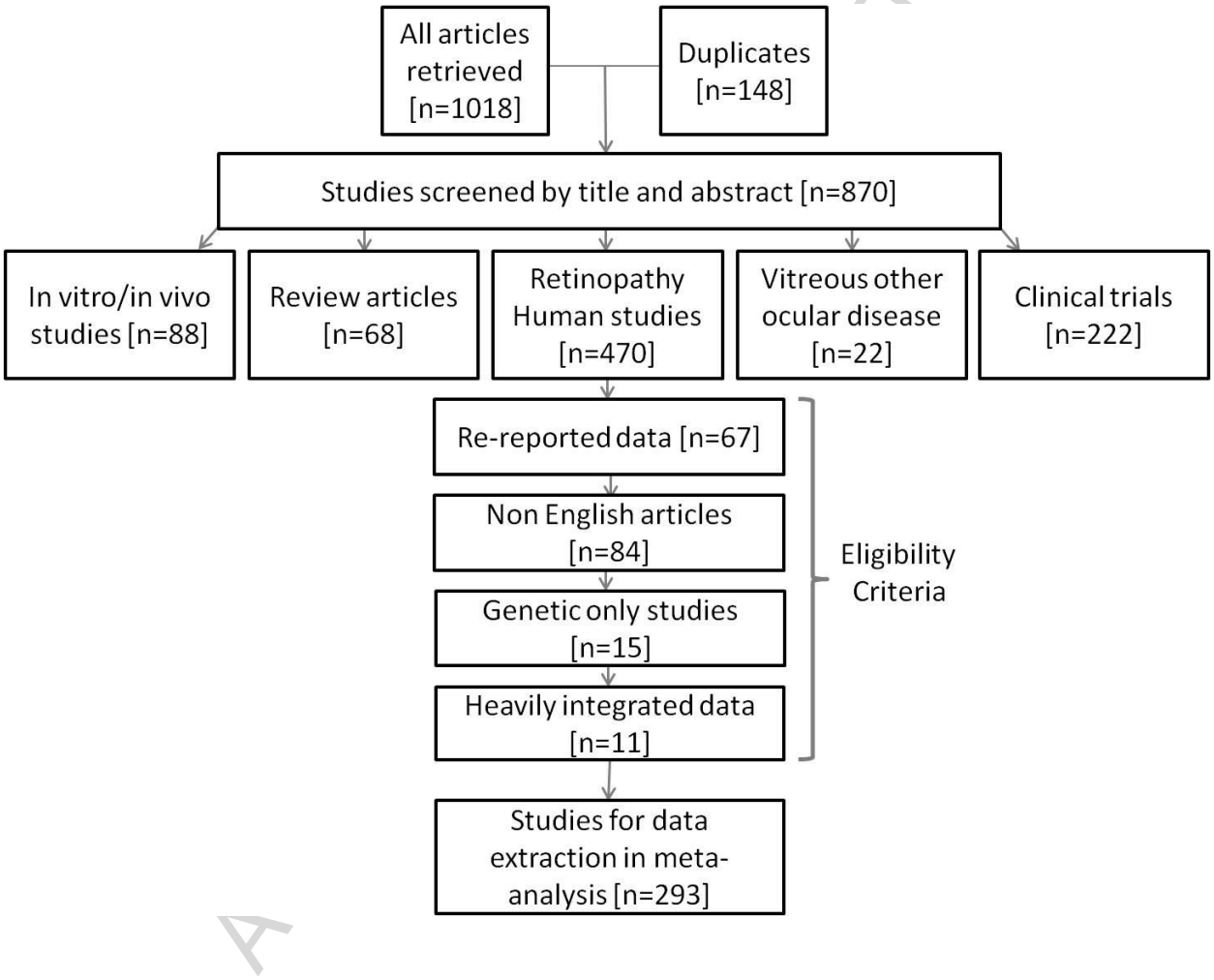
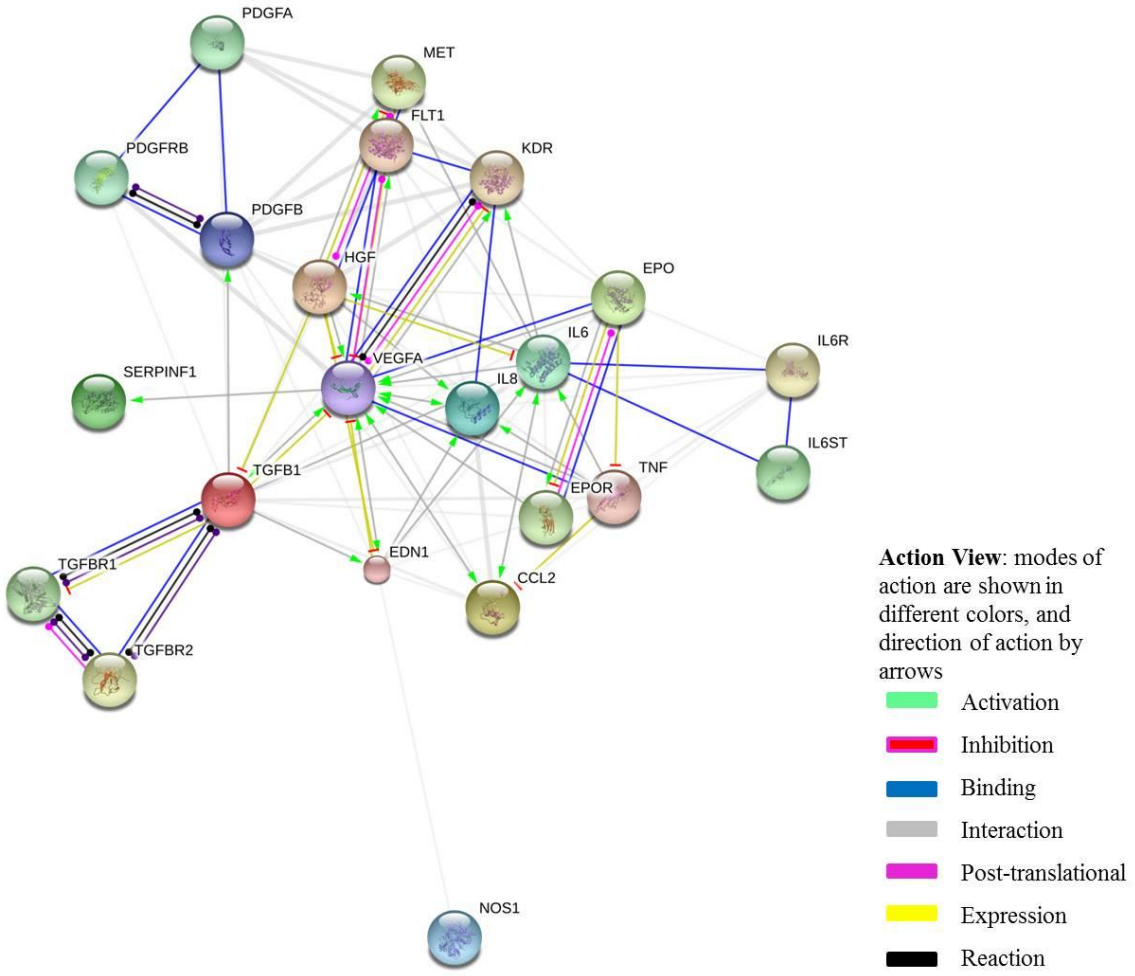


Figure 2. STRING pathway analysis of significant markers in proliferative diabetic retinopathy.



Abbreviations:
CCL2 (MCP-1): monocyte chemotactic protein 1, EDN1 (ET-1): endothelin-1, EPO: erythropoietin, EPOR: erythropoietin receptor, FLT1: vascular endothelial growth factor receptor 1, HGF: hepatocyte growth factor, IL6: interleukin-6; IL8: interleukin-8, IL6R: interleukin-6 receptor, IL6ST: interleukin-6 receptor subunit beta, KDR: vascular endothelial growth factor receptor 2, MET: hepatocyte growth factor receptor, NOS1 (NO): nitric oxide, PDGFA: platelet-derived growth factor subunit A, PDGRB: platelet-derived growth factor BB chain, PDGFRB: platelet-derived growth factor receptor beta, SERPINF1 (PEDF): pigment epithelium-derived factor, TGFB1 (TGF-β): transforming growth factor beta 1, TGFBR1: transforming growth factor beta receptor 1, TGFBR2: transforming growth factor beta receptor 2, TNF (TNF-α): tumor necrosis factor-alpha, VEGFA: vascular endothelial growth factor A.