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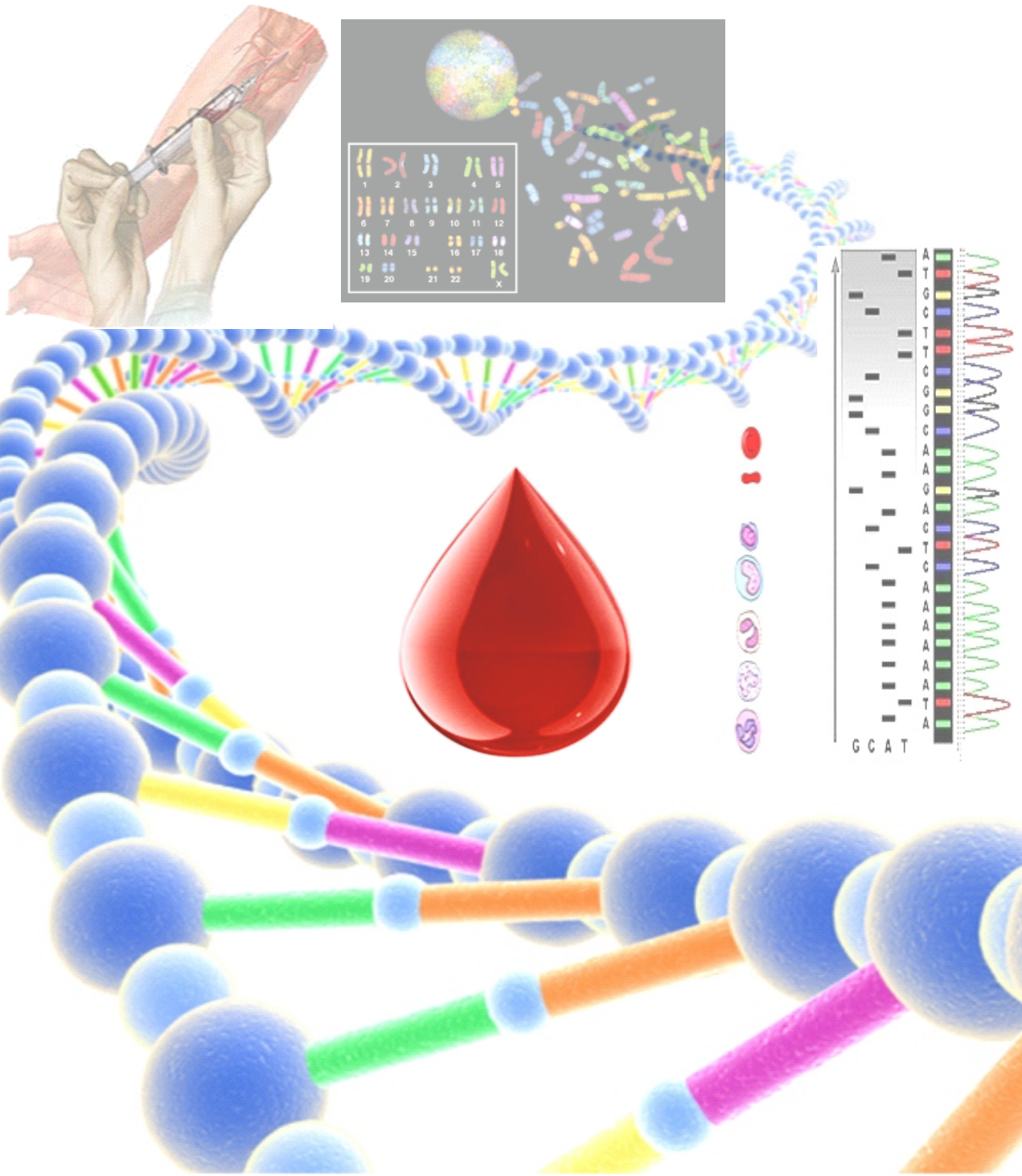

Cultural programme during the Foundation Day celebration of the Institute

Dr R Krishnamoorthy, INSERM, Paris, France Delivered 24th Dr H M Bhatia Memorial Oration

Training Programme on Transfusion Medicine at North East under ICMR Translational Research Programme

MATRIX METALLOPROTEINASES (MMPS) IN HUMAN HEALTH AND DISEASE

Ajay K. Chaudhary

Matrix metalloproteinases (MMPs) are a family of structurally related zinc-dependent endopeptidases, collectively capable of degradation of all components of extracellular matrix (ECM). It includes tumour growth, cancer progression, metastasis and angiogenesis. Over the last 10 years, the relevance of the MMP family in cancer research has grown considerably. As a result, these proteases have come to represent important therapeutic and diagnostic targets for treatment and detection of human cancer. But unfortunately, many cases the results derived from different sites of cancer for treating the patients with advanced cancer have been disappointing. Therefore, the purpose of this review was to clarify the inconsistent findings of the MMPs polymorphisms and its expression in solid and haematological malignancies.

1) Background

Basic cancer research is mostly paying attention on different gene mutations analysis in the different malignant lesions that result in either gain or lose of biological functions in the oncogenes and tumour suppresser genes respectively. However, stromal cells within tumours also play an important impact on malignant transformation, progression and initiation in different malignancies such as solid and as well as haematological malignancies.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases and their basic mechanism is to degrade the various components of the extracellular matrix (ECM) and basement membrane. MMPs plays an important roles in multiple physiological and pathological processes including development of the embryo, inflammation in the various stages, wound healing, angiogenesis, immunity, tumor invasion and metastasis (1-3). The expression levels of MMPs are increases in almost every type of human cancer and this correlates with advanced tumour stage. So, the many clinical data strongly support the participation of MMPs in the progression of human cancer.

Many study regarding the role and expression of MMPs and its tissue inhibitors (TIMPs) are well established in solid malignancies but in case of haematological malignancies only few studies has been done worldwide (4-7). Therefore, it is difficult to conclude the exact role of MMPs and its inhibitors in hematological malignancies with respect to their expressions, polymorphic association and its therapeutics aspects. However, the role of ECM-degrading enzymes, which are most probably produced by haemopoietic and stromal cells within the bone marrow microenvironment, has not been well understood.

2) Function and its regulation of MMPs

2.1. MMP structure, classification and function:

Jerome Gross and Charles Lapiere first described MMPs in 1962, who observed enzymatic activity in tadpole tail metamorphosis. MMP was purified from human skin by Eisen in 1968 (8). The MMPs have a common domain structure. The structure of MMPs includes the signal peptide domain, which guides the enzyme into the rough endoplasmic reticulum during synthesis, the propeptide domain, which sustains the latency of these enzymes until it is removed or disrupted, the catalytic domain, which houses the highly conserved Zn2+ binding region and is responsible for enzyme activity, the hemopexin domain, which determines the substrate specificity of MMPs and a small hinge region, which enables the

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hemopexin region to present substrate to the active core of the catalytic domain

Figure 1.

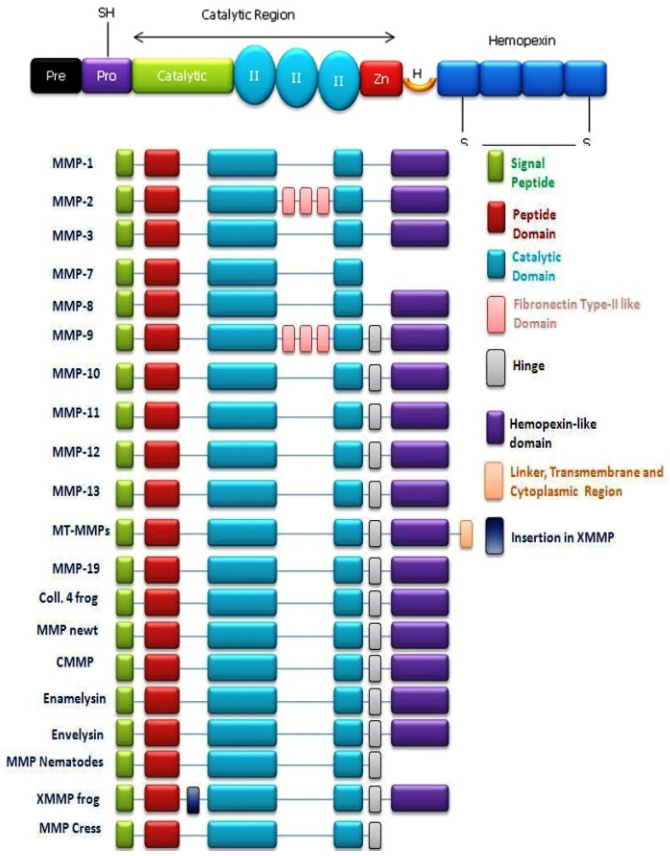

Fig. 1: [A] Basic domain structure of the gelatinases. The general structure of the MMPs includes a signal peptide, propeptide domain, catalytic domain with a highly conserved zinc- binding site and a haemopexin-like domain linked to the catalytic domain by a hinge region. [B] Systematic representation of structure of human matrix metalloproteinases. [Pre: Signal sequence, Pro: Pro-peptide with zinc-ligating thiol (SH) groups, II: Collagen-binding fibronectin type II inserts, Zn: Zinc-binding site, H: Hinge region, Hemopexin: The hemopexin domain contains four repeats with the first and last linked by disulfide bond].

Matrix metalloproteinase are a family of zinc (Zn+2) dependent endopeptidases that are capable of degrading various components of the ECM. Degeneration of matrix is the key event of the invasion and metastasis. Currently, 24 different types of MMPs have been identified among vertebrates, 23 of them have been found in humans (9-12).

The members of the MMP family have many similarities in their structure. All MMPs have a zinc-binding motif in the catalytic domain. In addition, they have an N- terminal domain called pre-domain, followed by the propeptide domain. The majority of MMPs also have additional domains, e.g., Hemopexin domain. These additional domains are important in substrate recognition and in inhibitor binding **(Figure1 A).** Based on their substrate specificity, MMPs have been divided into distinct subclasses: collagenases (MMP-1, MMP-8, MMP-13, and MMP-18), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP- 10 and MMP-11) and matrilysins (MMP-7, MMP-26) and other MMPs. The MMPs are inhibited by specific endogenous tissue inhibitors of metalloproteinases (TIMPs), which comprise a family of four protease inhibitors: TIMP1, TIMP2, TIMP3 and TIMP4. All types of MMPs have been described briefly in **Figure 1B &Table 1.**

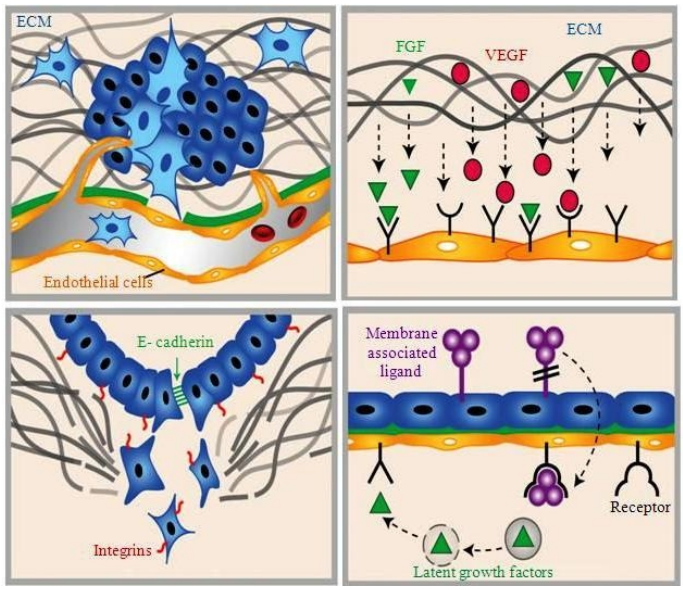

Figure 2: Multiple functions of matrix metalloproteases (MMPs) in cancer progression. MMPs degrade components of ECM, facilitating angiogenesis, tumor cell invasion and metastasis. MMPs modulate the interactions between tumour cells by cleaving E-cadherin and integrins which also enhances the invasiveness of tumour cells. MMPs also process and activate signaling molecules including growth factors and cytokines.

Table 1: Classification of vertebrate MMPs, their substrate and chromosomal location.

Types of MMPs	Common Name	Chromosomal Location	Substrates
MMP-1 MMP-8 MMP-13 MMP-18	Collagenase-1 Collagenase-2 Collagenase-3 Collagenase-4	11q22.2-22.3 11q22.2-22.3 11q22.2-22.3 Not in humans	Collagen II<I<III,VII,VIII,X,XI, Casein, perlecan, entactin, laminin, pro-MMP-1,2,9,serpins Collagen I>II>III>VII,VII,X,entactin,gelatin Collagen II>III>I,VII,X,XVIII,gelatin,entactin,tenascin,aggregan
MMP-2 MMP-9	Gelatinase-A Gelatinase-B	16q13 20q11.2-q13.1	Gelatin, fibronectin, elastin, laminin, collagen I,III,IV,V,VII,X,XI vitronectin, decorin Gelatin,CollagenI,IV,V,VII,X,XI,XVIII,vitronectin,Elastin,laminin,fibronectin
MMP-3 MMP- 10 MMP-11	Stromelysins-1 Stromelysins-2 Stromelysins-3	11q22.2-22.3 11q22.2-22.3 22q11.2	Laminin, aggregan gelatin, fibronectin CollagenI,III,IV,gelatin,elastin,proMMP-1,8,10 Fibronectin, laminin, aggregan, gelatin
MMP-12	Metalloelastase	11q22.2-22.3	Elastin, gelatin, collagen I,IV, fibronectin, laminin, vitronectin, proteoglycan
MMP-7 MMP-26	Matrilysin-1 Matrilysin-2	11q22.2-22.3 11q22.2	Collagen I,IV,V,IX,X,XI,XVIII, Fibronectin, laminin, gelatin, aggregan Gelatin, Collagen IV,proMMP-9
MMP-20	Enamelysin	11q22	Laminin, amelogenin, aggregan
MMP-14 MMP-15 MMP-16 MMP-17 MMP-24 MMP-25	MT1-MMP MT2-MMP MT3-MMP MT4-MMP MT5-MMP MT6-MMP	14q12.2 16q12.2 8q21 12q24 20q11.2 16q13.3	Collagen I,II,III,aggregan,laminin,gelatin,proMMP-2,13 Proteoglycans,proMMP-2 CoolagenIII,fironectin,proMMP-2 Gelatin,fibrinogen,proMMP-2 fibrinogen, Gelatin,proMMP-2 Collagen IV,gelatin,proMMP-2,9
MMP-19 MMP-21 MMP-22 MMP23 MMP-27 MMP28	Stromelysin-4 XMMP (Xenopus) CMMP (Chicken) Cysteine array CA-MMP Epilysin	12q14 - - 1p36.3 11q24 17q11.2	Collagen I,IV,Tenascin,Gelatin,Laminin Gelatin - Gelatin - Casein

Table 2: Role of MMPs and its inhibitors in haematological malignancies. [CLL= chronic lymphocytic leukemia, ICC= Immunocytochemistry, CA= Calorimetric assay, CALL= Childhood acute lymphoblastic leukemia, ICC=Immunocytochemistry

Authors name	Year of Publication	Country	Type of Cancer	Type of MMPs	Mode of analysis	Number of Case/Control	P Value
Casabonne et al (34)	2011	Spain	CLL	MMP-9	SNP	240/513	<0.002
Scrideli et al (35)	2010	Brazil	ALL	MMP-2,-9 & TIMP-1,-2	RT-PCR	134	<0.01
Schneider et al (36)	2009	France	ALL	MMP-2,-9,-14, TIMP-1,-2	ZA, FACS & ELISA	86	Significant
Dutta et al (37)	2010	India	CML	MMP-9	ELISA, RT-PCR, ICC	K562 Cells	Significant
Suminoe et al (38)	2007	Japan	ALL	MMP-2,-9 & TIMP-1,-2	RT-PCR	33	<0.005
Travaglino et al (6)	2008	Italy	AML & MDS	MMP-2,-9	ICC & CA	54 AML & 153 MDS	<0.0001
Iwata et al (39)	2007	USA	MDS	MMP-9	RT-PCR, IB, FISH	27	<.001
Lin et al (40)	2002	Taiwan	ALL, AML	MMP-2,-9 & TIMP-1,-2		18ALL, 37AML	Significant
Kuittinen et al (22)	2001	Finland	ALL	MMP-2,-9	ICC	75	12.7% positive
Ries et al (21)	1999	Germany	AML, CML & MDS	MMP-2 &-9	WB & ZA	AML=24, CML=17, MDS=8	Significant
Kuittinen et al (41)	1999	Finland	AML	MMP-2,-9	FACS	54	Significant

In most organs, the principle components of the ECM are collagens and numerous other proteins including laminin, entactin and proteoglycans that make up the basement membrane and extracellular matrix. Tumor cells over express the proteases and induces the expression of these enzymes in neighboring stromal cells in order to degrade the basement membrane and invade the surrounding tissue. Several MMPs have implicated in the ECM degradation, due to degradation, it is linked with tumor growth and angiogenesis. This proteolytic activity is also required for a cancer cell to invade a nearby blood vessel (intravasation) and then extravasate at a distant location to new metastatic site. So that MMP shows the multiple function of the cancer progression such as modulation of cell adhesion, migration, angiogenesis and metastasis.

2.2. Regulation of MMP expression and its activity:

MMPs are highly regulated proteins. The first level of regulation is transcriptional regulation. The basal gene-expression level and mRNA stability can be rapidly changed when remodeling of the extracellular matrix is required. This change can be achieved by change of ECM components or by growth factors of cytokines. Not all MMPs contain well-defined transcriptional elements; one of these MMPs is MMP-2. Formerly, it was thought that MMP-2 could only be post-transcriptional regulated, however, presently a number of well- known transcriptional elements have been identified in the MMP-2 promoter (13).

In general, the level of expression of MMPs is low in culture cells and in intact tissue in vivo. The expression of MMPs is induced by cytokines, growth factors, tumour promoters, oncogenic transformation, cell to matrix and cell to cell interaction. The expression of MMPs is primarily regulated at the level of transcription and proteolytic activity requires zymogen activation. Many stimuli increase the expression of c-fos and c-jun proto oncogene products and it's activate the activator protein-1 (AP-1) at proximal promoter regions of several MMPs such as MMP-1, -3, -7, - 9, -10, -12 and -13 types. Several oncogenes and viruses induce MMP expression in malignant cell lines (14). Activity of AP-1 element is mediated by three groups of mitogen-activated

protein kinases (MAPKs), which are mitogen-activated intracellular signal-regulated kinase 1, 2 (ERK1, 2), stress activated Jun N-terminal kinase and p38 MAPK (10). These three groups represent the MMPs regulation at the transcriptional and post transcriptional levels.

Based on the composition of cis-elements, the MMP promoters can be roughly grouped into three categories. The first group, including the majority of the MMP promoters, contains a TATA box at approximately -30 bp (relative to the transcription start site) and an AP-1-binding site at approximately -70 bp. Most of these promoters also contain an upstream PEA3-binding site that is often adjacent with an AP-1-binding site (15). The MMP promoters in the second group (MMP-8, -11 and –21) also contain a TATA box, but lack a proximal AP-1 site.

3) MMPs in human disorders:

3.1. Role of MMPs in haematological disorder:

During normal physiological process of the hematopoiesis, proliferation, differentiation and migration of the hematopoietic stem cells (HSCs) are regulated by their complex interactions with the surrounding bone marrow (BM) microenvironment, such as marrow stromal cells, cytokines and ECM proteins (16). Under steady-state conditions, mostly stem cells are maintained in G0 phase of cell cycle with BM stromal cells (17). Bergers et al reported that release of cytokines and growth factors from ECM membrane can alter the stem cells-stromal cells interaction and promote HSCs migration and differentiation (18). In normal BM tissue, MMP-2 and MMP-9 are detected in myeloid cells and megakaryocytes however; MMP-2 can also be detected in erythroblasts (19). In addition, membrane type MMPs (MT1-MMP, MT2-MMP, MT3- MMP and MT4-MMP) detected at different levels in erythroid, megakaryocytic and myeloid precursors expanded from normal BM CD34+ cells. These MMPs may play an important role in intercellular cross-talk in hematopoiesis (20).

Travaglino et al investigated potential role of MMP-2 and MMP-9 expression in myelodysplastic syndrome (MDS) and compared with acute myeloid leukemia (AML). They found that MMP expression in AML blasts were high as compared to MDS and suggested that the production and release of these enzymes may influence haematopoietic cell behavior (6). The molecular mechanism responsible for MMP over expression in dysplastic cells remains unknown neither it is clear whether MMPs are functionally active and play a role in the molecular pathogenesis of hematological malignancies worldwide. It may provide a useful tool for diagnosis, prognosis and as strategies for targeting MMPs as a new cancer treatment.

3.2. Expression of different MMPs and its inhibitors in hematological malignancies:

The main function of MMPs is the degradation of ECM. MMPs participate in the turnover of ECM in the hematopoietic microenvironment, regulating the release of hematopoietic stem cells and mature leucocytes from bone marrow (BM) to peripheral blood (PB). As in solid tumors, MMPs participate in ECM proteolysis and growth factor and cytokine release which are central part of the leukemia progression. The expression of MMPs, especially gelatinases A and B (MMP- 2 and MMP-9), was first described in adult acute or chronic myeloid leukemia (CML) and myelodysplastic syndromes (MDS) (21). Kuittinen et al found a remarkable difference in the expression level of MMP-2 and -9 between pediatric and adult ALL cases; in adult ALL patients 65% of the cases showed positive staining in blast cells for MMP-2 and 25% for MMP-9 (22). Low MMP-9 levels at diagnosis of AML suggest that the lack of MMP-9 can eventually play a role in leukemic progression. Arimura et al examined the involvement of apoptosis with MDS accompanied by peripheral cytopenias and

suggested the MMPIs inhibits the apoptosis induction of MDS in bone marrow cells (23). Suminoe et al studied the correlation of MMP and TIMP gene expression in leukemia cells with clinical characteristics of infants with ALL and reported that mRNA contents of MMP-2 and MMP-9 were not associated with any patient characteristics (24). Recently, Schneider et al analyzed the expression of MMP-2, -9, -14 and TIMP-1 and -2 in a childhood acute lymphoblastic leukemia by flow cytometry and production of MMPs were quantified by ELISA and zymography. They showed that high secretion of MMP-9 was associated with a lower overall survival rate and suggested that the secretion of MMP-9 is an independent prognostic factor in childhood B-ALL (25). Many researchers have suggested that MMP-9 expression might be involved in physiological processes, e.g. the differentiation process in hematopoiesis (Table 2). However, till date, the differences in MMP-9 expression between MDS, AML, CML and normal bone marrow cannot be explained by the maturation stage of the leukemic cells in the bone marrow.

3.3. Single nucleotide Polymorphism and expression analysis of the MMPs in solid cancer:

Metalloproteinase and its tissue inhibitor take part in the degeneration of the extracellular matrix and are associated with carcinogenesis in head and neck lesions. MMP-1 (Collagenase-1) is a major proteinase of the MMP family that specifically degrades type I collagen, which is a major component of the ECM, as well as other fibrillar collagens of types II, III, V and IX. The MMP-1 gene is located on chromosome 11q22 and the level of expression of this gene can be influenced by SNPs in the promoter region of their respective genes. Rutter et al suggested that a single nucleotide polymorphism at -1607 bp in the MMP-1 promoter contributes to increased transcription and cells expressing the 2 G polymorphism may provide a mechanism for more aggressive matrix degradation, thereby facilitating cancer progression (26). The promoter region of MMP-1 contains a guanine insertion/ -1607 and assays have indicated that this is a functional polymorphism. Tower et al reported that this 2G allele results in increased transcriptional activity because the guanine insertion creates a core-binding site (5'-GGA- 3') for the Ets transcription factor family, leading to a higher expression of MMP-1 (27). Globally, several authors have been reported significant association of SNPs with different types of MMPs gene **(Table 3)**.

A subgroup of MMPs, the gelatinases (MMP-2 and MMP-9), have been particularly implicated in progression, angiogenesis and metastasis of various cancer types. MMPs play an important role in colorectal cancer (CRC). Park et al concluded that specific single-nucleotide polymorphism (SNP) in TIMP-2 and MMP-9 appeared to be associated with tumorigenesis and biological behavior in colorectal cancer (28). Association of SNP at MMP-1 16071G/2G, MMP-7 181A/G and MMP-9 279R/Q genes with CRC in the southwest Chinese Han population suggested that there was no significant differences in the genotype and allele frequencies between the case and control groups (29).The MMPs plays a role in regulation of cell growth, apoptosis, angiogenesis and immune surveillance. Natural sequence variations in the MMP genes may result in differential expression of MMPs in different individuals and therefore may be associated with the development and progression of these disorders. Wang et al studied the effects of the MMP-9 (-1562 C/T) promoter polymorphism on the risk of occurrence and lymphatic metastasis in 243 non-small cell lung carcinoma (NSCLC) Vs 350 healthy controls and suggested that the MMP-9 (-1562 C/T) polymorphism may not be used as a useful marker to predicate susceptibility and lymphatic metastasis in NSCLC (30).

Single nucleotide polymorphisms (SNPs) of MMPs gene promoter may alter MMPs protein expression levels to influence malignant tumors developing and progressing. Jia et al investigated whether the functional polymorphisms in the promoter region of MMP-12 (-82A/G) and MMP-13 (-77A/G) are associated with epithelial ovarian carcinoma (EOC). They reported that -82A/G and -77A/A genotype frequencies of the MMP-12 and MMP-13 genes respectively was significantly higher in the patients than in the controls (P= 0.003) (31). These results along with others (32) suggest that individuals with MMP-12 -82A/G and MMP-13 -77A/A might have higher risk of overall or special histological type of EOC development.

4. Involvement of MMP-inhibition in cancer therapy

Last 50 years, many pharmaceutical industries have focused the enormous work in the oncology field for the development of the noble drugs and it has been made in the treatment of selective types of cancers such as leukemias, lymphomas, testicular, lung and gastrointestinal cancer. Invernizzi et al treated with primary MDS patients with thalidomide and observed that significant reduction of blood transfusion and bone marrow blasts. After treatment of MDS patients, expression level of MMP-2 and -9 decreases in bone marrow cells and also observed a reduction of CD4 cells and increase of NK cells in peripheral blood (33). Therefore, they concluded that thalidomide may produce a fairly good hematological improvement in erythroid cells in MDS patients.

Many MMP inhibitors (MMPIs) have been in clinical trials and are expected to present a new approach to cancer treatment. MMPIs may inhibit malignant growth by enhancing fibrosis around malignant lesions, which in turn prevents tumour invasion, apoptosis and angiogenesis. Inhibitors of MMPs fall into five categories: (A) Peptidomimetics (B) Nonpeptidomimetics (C) Natural MMPIs (D) Tetracycline derivatives and (E) Bisphosphonates. Batimastat (BB-94) is a low molecular weight peptidometric hydroxamate based inhibitor that inhibits MMPs. It was the first MMPIs evaluated in cancer patients and to be used in a clinical trial. BB-94 inhibits the activity of MMP-2 and MMP-9.

Marismastat (BB-2516) is a synthetic low molecular weight (331.4 D) peptidomimetic MMPI. It inhibits the genomic and proteomics activity of MMP-1, MMP -2, MMP -3, MMP -7, MMP -9 and MMP -12. The drug contains a collagen-mimicking hydroxamate structure that chelates the zinc ion at the active site of MMPs. Marimastat has been studied in phase II trials in patients with colorectal and advanced pancreatic cancer and also in phase III clinical trials for treatment of pancreatic, ovarian, gastric and breast cancers as well as squamous cell lung carcinoma (SCLC) and non-squamous cell lung carcinoma (NSCLC). Overall survival of patients with advanced pancreatic cancer who were treated with Marimastat was not better than that of patients treated with Gemcitabine.

Table 3: Functional polymorphism of MMPs in lesions of the solid malignancies [PCR-RFLP = Polymerase chain reaction-fragment length polymorphism, ESCC = Esophageal squamous cell carcinoma, OSCC = Oral squamous cell carcinoma, OSF = Oral sub-mucous fibrosis, NPC = Nasopharyngeal carcinoma, CRC= Colorectal cancer, HNSCC = Head and neck squamous cell carcinoma, TC = Tongue Cancer, S= Significant, NS = Not significant, TC=Tongue cancer, CC=colorectal cancer, LC=lung cancer, EOC=epithelial ovarian carcinoma, OC= Ovarian cancer, CC= Cervical cancer]

Authors name	Publication Year	Country	Type of Cancer	Type of MMPs	Types of Polymorphism	Mode of analysis	Case/Control	OD (95%CI)	P Value
Chaudhary et al (42)	2011	India	OSMF HNSCC	MMP-2 MMP-9	-1306 C/T & -168 G/T -1562 C/T	PCR-RFLP	412/426 422/426	1.86 (1.15–1.85) 1.17 (1.06–1.79)	< 0.00 <0.01
Malik et al (43)	2011	India	ESCC	MMP-7	-181A>G	PCR-RFLP	135/195	2.17 (1.21-3.92)	<0.010
Chaudhary et al (44)	2010	India	OSMF HNSCC	MMP-1	-1607 1G/2G	PCR-RFLP	412/426 422/426	5.07 (2.827–9.115) 3.53 (1.892–6.547)	<0.001 <0.00
Chaudhary et al (3)	2010	India	OSMF HNSCC	MMP-3	-1171 5A->6A	PCR-RFLP	101/126 135/126	2.26 (1.22-4.20) 1.94 (1.06-3.51)	<0.01 <0.03
Bradbury et al (45)	2009	Canada	EC	MMP-1 MM-3 MMP-12	-1607 2G>1G -1171 5A>6A -82A/G & 1082A/G	PCR-FLP	313/455	1.46 (1.0-2.1) 1.61 (1.0-2.5) 1.36 (1.0-1.8) 1.70 (1.1 2.6)	< 0.04 < 0.03 < 0.03 < 0.01
Vairaktaris et al (46)	2008	Greece	OSCC	MMP-9	-1562 C/T	PCR-RFLP	152/162	1.92 (1.21-3.06)	<0.05
Shimizu et al (47)	2008	Japan	TC	MMP-1 & IL-8	-1607 2G/2G -251 A/A	SNPs	- -	- -	NS < 0.0068
Nasr et al (48)	2007	Tunisia	NPC	MMP-1 MMP-9	-1607 2G>1G -1562 C/T	PCR-RFLP	174/171	2.27	< 0.02 NS
Singh et al (49)	2008	India	CC	MMP-7	-181A>G	PCR-RFLP	150/162	1.94	0.041
Park et al (50)	2011	Korea	CRC	MMP-2,-9 &TIMP-2	-1575*G/*G, -418*G303*G	PCR-RFLP	NA	-	significant
Fang et al (51)	2010	Chaina	CRC	MMP-1 MMP-7 MMP-9	16071G/2G 181A/G 279R/Q	PCR-RFLP	237/252	- - 1.74 (1.323-2.281)	NS NS < 0.001
de Lima et al (52)	2009	Brazil	CRC	MMP-1 MMP-7	-1607ins/delG -181A/G	PCR-RFLP	130/130	6.45 (2.02-20.6) -	<0.001 NS
Kouhkan et al (53)	2008	Iran	CRC	MMP-1	-1607 1G/2G	PCR-RFLP	150/100	-	Significant

5. Future perspectives:

Many studies have efficiently shown that several types of MMPs contribute in solid and haematological malignancies. MMPs promote cancer progression by increasing cancer-cell growth, migration, invasion, metastasis and angiogenesis. The balance between MMPs and TIMPs is critical for the proper maintenance of functional homeostasis in hematopoietic tissue and also in solid tumors. A number of MMPs play important roles in physiological hematopoiesis. However, most studies on the roles of MMPs in hematological malignancies have been focused on their effects on the invasion and transmigration behavior of the different types of leukemia cells. MMPs may also take part in pathological BM angiogenesis and leukemic cell survival through growth factors release, which is promoting leukemia progression. Therefore, it is essential to elucidate the complex effects of MMPs/TIMPs and the biological roles of MMPs in pathological BM angiogenesis and leukemic cell survival and growth.

Several synthetic MMP inhibitors are undergoing Phase II and III clinical trials. Although a few encouraging results have been reported, some trials were prematurely terminated due to either lack of benefits or major adverse effects. The clinical trials

have so far focused on patients with advanced-stage disease. At present, bio-available oral MMPIs have been designed and tested clinically for the treatment of different types of solid malignancies but not in haematological malignancies.

Several MMPIs have shown anti- leukemia activity in vitro and in vivo. So far, none of the MMPIs has been clinically tried in hematological malignancies due to the limited understanding about the exact roles of MMPs in these diseases. In addition, the application of MMPIs in hematological malignancies may face more problems in comparison with that in solid tumors. Since MMPs are key regulators in physiological hematopoiesis, any overcorrection may lead to a negative effect. Therefore, future studies should first elucidate the deregulation of different MMPs production by different BM subsets in hematological malignancies pathogenesis and then identify specific MMP targets to improve anti-leukemia efficacy. Moreover, it's likely that MMPIs alone are insufficient in treatment of late stage hematopoietic diseases. The application of one or more selective-targeted MMP inhibitors in combination with conventional anti-leukemia treatment may represent a promising strategy in the combat against hematopoietic malignancies.

6. Conclusions:

Over the last 10 years, the relevance of the MMP family in cancer research has grown considerably. Unfortunately, many cases the results derived from different sites of cancer using broad-spectrum MMPIs for treating patients with advanced cancer have been disappointing. Therefore, the purpose of this review was to clarify the inconsistent findings of the MMPs polymorphisms and its expression in solid and haematological malignancies. This review concluded that molecular markers might be plays as a vital role in predicting the different malignancies. Furthermore, the introduction of novel prognostic markers might promote exclusively new treatment possibilities in early stage.

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RAPID FLOW CYTOMETRIC TEST USING EOSIN-5 '-MALEIMIDE DYE FOR THE DIAGNOSIS OF RED BLOOD CELL MEMBRANE DISORDERS

Prabhakar S. Kedar

Hereditary spherocytosis (HS) is one of the most common hereditary anemias. Clinical manifestations of HS are extremely variable, ranging from asymptomatic patients to severely anemic, transfusion-dependent patients. HS is caused by genetic defects of spectrin, ankyrin, band 3 proteins, protein 4.2 and/or other erythrocytes plasma membrane proteins. Deficiency of cytoskeleton proteins results in the characteristic red blood cell (RBC) shape [1]. Increased osmotic fragility (OF) of spherocytes is a result of impaired proportions between the surface area and volume of the cells. These RBCs hemolyze in hypertonic environments and can be further damaged during splenic conditioning. This test gives false positive in many hematological disorders. Diagnosis of red cell membrane protein disorders, particularly HS is based on clinical features, family history, and peripheral smear examination, along with other commonly used laboratory investigations such as osmotic fragility, the autohemolysis test and the acidified glycerol lysis test. As these methods for diagnosis of hereditary red blood cell (RBC) membrane disorders, in particularly hereditary spherocytosis (HS), is laborious, time consuming and requires at least 2 ml of blood, which might be impractical in neonatal period [2]. More advanced methods, only used in reference centers include sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of RBC plasma membrane proteins and molecular diagnosis of mutations in integral proteins of erythrocytes membrane coding gene [3]. These methods require a lot more skill to perform, are time consuming and unavailable in routine hematological laboratories, while other screening tests for HS do not have high specificity and sensitivity.

Recently, a flow cytometric method for diagnosis of HS has been described for quantitating the fluorescence intensity of intact red cells after incubation with the dye eosin-5′-maleimide (EMA) [4]. We have also been experienced the use of eosin-5-maleimide (EMA) dye test and was found to be an effective screening test for HS [5]. EMA dye reacts covalently with lysine-430 on the first extracellular loop of band 3 proteins and an advanced rapid screening test for the patients with HS, Herediatry elliptocysis (HEE) and Southeast Asian Ovalocytosis (SAO). RBCs from patients with HS and SAO expressed a greater degree of reduction in MCF compared to those from normal controls and other hemolytic diseases [4-6]. Our findings showed that this flow cytometric-based method is a simple, sensitive and reliable diagnostic test for RBC membrane disorders using a small volume of blood, and the results could be obtained within 2 hours. Such method could be serve as a first line of screening for the diagnosis of HS and SAO in routine hematological test before further specific membrane protein electrophoresis and molecular diagnosis are employed. This eosin-5 ′ -maleimide (EMA) staining test is an advanced flow cytometric test and widely used as screening for the diagnosis of hereditary spherocytosis (HS) [4-6].

Flow cytometric analysis of EMA-labeled red blood cells

Five microliters of blood is washed with phosphate-buffered saline containing 0.5% fetal bovine serum and is incubated for 1 hrs with 25 µl of EMA dye (0.5 mg/ml in PBS, Fluka, Gillingham, UK) in dark at room temperature with intermittent mixing. The stock of EMA solution can be stored up to one month at

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–20°C or up to four months at –80°C. RBCs after incubation are centrifuged at 8500g for 30 sec. Supernatant with unbound dye is removed carefully. RBC pellets are then washed three times with 0.5 % FBS/PBS and centrifuge each time for 30 sec at 8500 g in a microcentrifuge. Washed cells are resuspended in 500 µl of 0.5% FBS/PBS. One hundred microliters of labeled cells are resuspended in 1.4 ml 0.5 % FBS/PBS for flow cytometric analysis. Intensity of dye fluorescence is determined in the FL-1 channel for 20,000 events on a FACS caliber flow cytometer (Becton Dickinson, San Jose, CA, USA). RBC are gated in the A region on the FS log/SS log dot plot. Fluorescence analysis is performed for the A region. The flow cytometer is standardized against Flow-set fluorospheres (Becton Dickinson) before use.

Interpretation

The results of flow cytometric analysis of EMA labelled red cells is expressed in mean channel flurorescence (MCF). Each laboratory should establish their reference normal range using healthy normal control and also for clinically diagnosed hereditary spherocytosis patient. Every patients sample should be assay simultaneously with normal control to give definite diagnosis. The control group of hematologically normal adults in our laboratory showing normal ranges 900 to 1200 MCF. The MCF of the HS cases is fall within range 650 to 900 MCF, which is significantly lower as shown in fig-1.

Troubleshooting

The few reports using this approach have shown that the EMA MCF values in normal control patients are quite variable. King et al (2000) reported a mean ± SD of 53.91 ± 3.24 in normal adults [4], while Fisher et al (2002) reported a mean EMA FU ± 2 SD of 599.7 ± 85.4 for a similar group [7] (Table-1). In our previous study, we found a mean ± SD of 288 ± 28 in healthy adults. Hence, it is important that each laboratory should establish its own reference normal range. The reproducibility of EMA fluorescence measurements depends on the storage conditions of the dye, and we studied the dye stability during storage in the dark at three different temperatures, 4°C., −20°C., and −80°C. It can be seen that there was a rapid decrease in MCF when the dye was stored at 4°C. However, the MCF of intact red cells labeled with EMA remained unchanged when the dye was stored at −80°C. Storage of the dye at −20°C also resulted in some reduction in MCF over a 4-month period [5].

Advantages

The EMA binding test is very useful as only a small volume of blood is required, and the result does not depend on the patient's age and does not change after splenectomy. This flow cytometric dye method is a reliable diagnostic test for RBC membrane disorders as well as being a simple, user-friendly and rapid method which is inexpensive, provided a flow cytometer is available. It is speedy diagnostic test (2 hrs from sample collection to result) for HS with a high sensitivity.

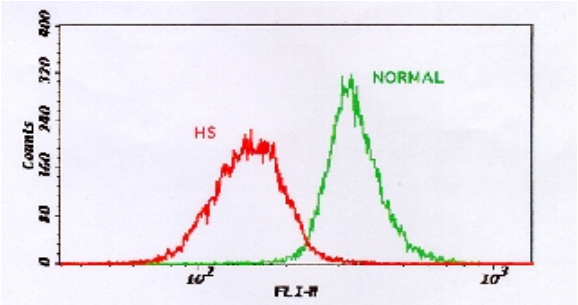

Figure 1. Histogram showing distribution of mean channel fluorescence for normal control and patient groups. Green histogram is observed in hematologically normal adults; cord blood samples; glucose-6-phosphate dehydrogenase (G6PD) deficiency; pyruvate kinase (PK) deficiency; β-thalassemia trait; sickle cell disease; autoimmune hemolytic anemia. Red colour histogram is observed only in hereditary spherocytosis and hereditary elliptocytosis cases.

Table 1. Flow cytometric analysis of eosin-5′-maleimide (EMA) labelled red cells (mean channel fluorescence, MCF units) obtained in different hematological laboratories.

Sr No	Normal Controls MCF units (mean ± SD)	Hereditary Spherocytosis MCF units (mean ± SD)	Reference
1	288 ± 28	202 ± 27	Kedar et al (2003)
2	53.91 ± 3.24	43.3± 3.4	King MJ et al (2000)
3	599.7 ± 85.4	480 ± 38	Fisher J et al (2002)
4	11 861.5 ± 883.5	7949.3± 1304.1	Kar R et al (2011)
5	400.0± 27.5	280± 43	Stoya et al (2006)
6	1176± 69	831 ± 80	Recently established

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LIBRARY INFORMATION SCIENCE

General Information

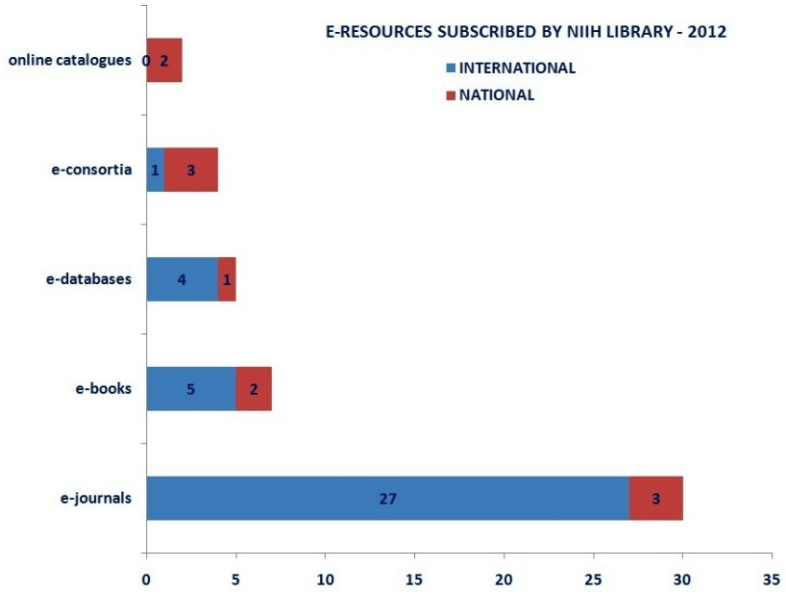
Revolution in Information Communication Technology (ICT) in the past couple of decades had drastic and far-reaching influences on all aspects of human life. Digital Libraries and knowledge management are the key zones that are still coming up in the developing countries and have a great potential to become an important technology in creative knowledge and its management.

The Digital Libraries are electronic libraries where the information is acquired, stored and retrieved in digital form. Digital Libraries are the group-interlinked workstations connected to the high-speed networks. In digital libraries the e-resources are mounted on the

servers located at remote places and the member libraries using user login and password on yearly subscription basis. The digital collection resources may be e-journals, bibliographic and full text databases, e-books, e-prints etc. which are presented in the digital collection and catalogue of the member libraries for a particular period of time. Many libraries may use the digital collection available at one site at the same time without any problems over the Internet through Dial Up, DSL, Lease Line or VSAT connectivity. So the digital libraries are libraries without wall and it is also known as virtual library in bigger sense. Library collection in digital form can be shared, exchanged and accessed by the members of the library and information network libraries as per their contracts. An important feature of the Digital object is that it can be stored, processed and played back using a computer system, network and internet. It allows us to search for any keywords of phrase or subject descriptor in the entire collection. It also occupies less space; require less manpower to run and manage the digital library, therefore it is very cost effective.

Our library is planning to go for digitalization of the library resources. Presently library subscribes to 27 International and 3 National e-journals, 7-ebooks on Hematology, Immunology, Blood Transfusion, Hematogenetics, Haemostasis, Genetics etc.

The Library has also subscribed to various e-databases of Thomson Reuters, J-Gate, JCCC@ICMR full-text database, NML-ERMED consortia, the Cochrane Library and ICMR e-journals consortia.



Facilities :

Printer for poster, colored scanner and Photocopier.

Online full text of Journals (IP oriented) accessible through Wi-Fi to students and staffs.

Electronic transfer of Full-text articles i.e. Inter-library loan facility to nearby medical colleges and research institute libraries.

Publications

Annual Report of the Institute for the year 2011-2012.

Some Recent Additions (Apr .2011-Mar. 2012)

Books	-	67
Journals	-	International- 40
		National-12
Bound Volumes	-	Nil
CD-ROM/DVD	-	40

NIIH HAPPENINGS

Scientific Meetings

Dr. K. Ghosh, Director:

1. Attended the Director's meeting at Jodhpur from 13th -14th January 2012.
2. Attended the Brain Storming meeting on "A multi centre comparative study for estimation and method" at ICMR, New Delhi on 20th January 2012.
3. Attended the Annual meeting of Hemophilia Federation of India, at New Delhi from 21st-22nd January 2012.
4. Attended the Meeting of South Asian Forum for Health Research at New Delhi from 5th-7th February 2012.
5. Invited as a Expert to the Span Technical Committee Meeting at Surat on 18th February 2012.
6. Attended the Annual Research Meeting of Hinduja Hospital, Mumbai on 31st March 2012.
7. Attended the Jai Vigyan Project Phase II meeting at St.John's Hospital, Bangalore from 26th-27th April 2012.
8. Attended the Tribal Health Forum Meeting at RMRC Port Blair, from 2nd-3rd June 2012.
9. Attended the Color Scale meeting at Dept. of Hematology, AIIMS, New Delhi on 20th June, 2012
10. Attended the Task Force Meeting on Extra hepatic Portal Venoms at ICMR, New Delhi on 19th July 2012.

Dr. Roshan Colah, Scientist F:

1. Attended the Translational Research Group meeting at ICMR Headquarters, New Delhi on 5th March, 2012.
2. Invited to attend the Work group meeting on National Task Force for - Thalassemia Control in Delhi and Punjab on 15th March, 2012 at ICMR Headquarters, New Delhi.
3. Attended the Bio-rad Uuser's meeting on 27th April, 2012 at Mumbai.
4. Attended the Red Cross Blood Transfusion Sub-Committee meeting on 30th April, 2012 at Mumbai.
5. Attended the Tribal Health Research Forum meeting at RMRC, Port Blair from 2nd - 3rd June, 2012.
6. Attended the Red Cross Blood Transfusion Sub-Committee meeting on 19th June, 2012.
7. Attended a meeting of the Tribal Health Research Forum to discuss the projects on Sickle cell and G6PD deficiency on 12th July, at ICMR, New Delhi.
8. Attended a steering committee meeting of the Jai Vigyan Project at ICMR, New Delhi on 30th July, 2012.
9. Attended the Tribal Health Research Forum meeting at RMRC, Bhubhaneshwar from 8th - 9th August, 2012

Dr. Malay Mukherjee, Scientist D:

1. Attended Tribal Health Forum Meeting held at ICMR, New Delhi on 8th May 2012.
2. Attended Condemnation Committee Meeting held at ICMR, New Delhi on 14th May 2012.
3. Attended Tribal Health Forum Meeting held at RMRC, Port Blair from 2nd to 3rd June 2012.
4. Attended Tribal Health Forum Meeting held at RMRC, Bhubaneshwar from 8th to 9th August 2012.

Conferences/Seminar/Workshops:

Dr. K. Ghosh, Director

1. Delivered the Inaugural lecture at the DRDO Meeting on "Hematology Basic and Current

Trends" at New Delhi on 30th January 2012.

2. Attended the CME for Hemophilia Therapy at Aurangabad on 25th February 2012.
3. Delivered a lecture on "Treatment of Transfusion Management of Thalassaemic Patients" at Agartala, Tripura on 26th June, 2012.
4. Attend the XXX international Congress of the World Federation of Haemophilia-2012 at Paris from 8th-12th July, 2012.

Dr. Roshan Colah, Scientist F:

1. Invited to give a talk on "Complexities in prenatal and postnatal diagnosis of hemoglobinopathies - Lessons learnt" on 28th January, 2012 at Bombay Hospital, Mumbai.
2. Invited to give a talk at the 1st Pan Asian Conference on Hemoglobinopathies on "Control Strategies for Thalassemia in India" held at Bangkok from 8th to 10th February, 2012.
3. Invited to deliver a talk on "Hemoglobinopathies" at the Annual Pediatric Genetics (PediGen) Conference, Pune chapter on 12th February, 2012 at Deenanath Mangeshkar Hospital, Pune.
4. Attended an Workshop on "Molecular Analysis & Prenatal Diagnosis of Thalassemia Syndromes" under the Translational Research Programme was organized at VCRC, Pondicherry from 24th to 26th April, 2012.
5. Invited by Foundation for Medical Research and delivered a talk on "Inherited Disorders of Hemoglobin: Epidemiology and Control" on 27th June, 2012 at Worli, Mumbai.

Dr. Manisha Madkaikar, Scientist E:

1. Invited to deliver a lecture on 'Advances in Autoimmune lymphoproliferative Syndromes' 'XVIIIth National CME in Haematology & Haemato-Oncology' held at Bombay Hospital on 26th Jan 2012.
2. Invited to deliver a lecture on 'PID in India: Mumbai Experience' at 1st National Primary Immunodeficiency Disorders Conference held at Chandigarh from 10-11th March 2012.
3. Presented a paper entitled "Comprehensive evaluation of NK-cell functions in patients with Hemophagocytic lymphohistiocytosis (HLH)" in the 1st National Primary Immunodeficiency Disorders Conference held at Chandigarh from 10-11th March 2012.
4. Invited to deliver a lecture on 'Laboratory Approach in PID' at CME on Primary Immunodeficiency Disorders held at Bhubaneswar, Orissa on 8th April 2012.
5. Invited as speaker for '9th Clinical Cytometry Course' conducted at Tata Memorial Hospital, Mumbai on 8th Aug 2012 and delivered a lecture entitled "Flowcytometric diagnosis of PNH".

Dr. Shrimati Shetty, Scientist E

1. Invited to deliver the following two lectures in the XVIII National CME in Haematology held at Bombay Hospital, Mumbai from 26th to 29th January 2012.
   - Inhibitor development- Risk factors
   - Microparticles in health and disease
2. Invited to deliver a lecture on "Preservation & storage of platelets" at S.L.Raheja Hospital, Mahim, Mumbai organized by Federation of Bombay Blood Banks on 3rd February 2012.

Dr. Malay Mukherjee, Scientist D:

1. Attended International Conference on Genes, Genetics and Genomics: Today and Tomorrow-Human concern and 37th Annual Conference of Indian Society of Human Genetics" held at Punjab University from 3rd to 5th March 2012 and presented a paper entitled "Genetic polymorphisms of the UDP glucuronosyltransferase (UGT1A1) gene and their influence on serum bilirubin levels in healthy Indian adults".

Dr. V. Babu Rao, Scientist D:

1. Attended International conference on "Genes,

Genetics & Genomics: Today & Tomarrow- Human Concerns & 37th Annual conference of the Indian Society of Human Genetics" held at Panjab University, Chandigarh from 3-5th March, 2012.

2. Invited to deliver a lecture on "Genetics and Humn Health" for Ph.D and MD students at Seth G.S Medical College & K.E.M Hospital on June 29th 2012.

Dr. Aruna Shankarkumar, Scientist C:

1. Delivered a lecture during the EQAS workshop, organized by the Department of Microbiology, NAIR Hospital on 8th May 2012.
2. Attended the Laboratory Management and Internal Audit training: ISO 15189 2007, organized by Department of Microbiology, KEM Hospital from 9th – 12th May 2012.
3. Organized the EQAS Panel distribution workshop for linked SRLs on 7th August 2012.

Dr. Swati Kulkarni, Scientist B:

1. Attended a training programme on "The Science of Living" conducted by Academy of human excellence under the scheme "National programme for training of women scientists & Technologists working in the Govt. Sector' sponsored by DST from 9th-13th January 2012.
2. Presented a Poster entitled "Detection of red cell bound immunoglobulin's by Enzyme Linked Antiglobulin Test and Flowcytometry", in the 35th Annual conference of Mumbai Haematology Group on 3rd-4th March, 2012.
3. Invited as panelist in discussion on "Newer trends in transfusion medicine - Partial D in the 35th Annual conference of Mumbai Haematology Group from 3rd - 4th March, 2012.

Dr. Bipin Kulkarni, Scientist B:

1. Invited as a Guest Speaker and Teaching faculty for a Workshop on "Blood Coagulation Techniques for the diagnosis of bleeding disorders" held at Apollo Hospital, Bhubaneswar on 12th February 2012.

Dr. P S Kedar, Technical Officer:

1. Presented a Paper entitled "NADH-cytochrome b5 reductase deficiency: an unusual cause of Recurrent Early Pregnancy Loss (REPL) in an Indian family" at International Conference on Genes, Genetics & Genomics: Today & Tomorrow- Human Concerns and XXXVII Annual Conference of The Indian Society of Human Genetics was held at Punjab University, Chandigarh from 3rd -5th March 2012.

Dr. Mehul S. Rajpurkar, TO, NACO Project

1. Attended the Quality Manual Writing and SIMS Workshop organized by NACO at Pune from 27th – 29th August 2012.
2. Attended the EQAS Panel distribution workshop organized by NARI, Pune on 30th August 2012.

Maya Gupta, Technical Assistant

1. Presented a paper entitled "Prenatal diagnosis of Primary Immunodeficiency Disorders by flowcytometry" in the 1st National Primary Immunodeficiency Disorders Conference held at Chandigarh from 10th -11th March 2012.

Sharda Shanbhag, Technician C

1. Presented a paper entitled "Molecular basis of factor XIII deficiency; seven novel mutations detected" in the Annual Conference of Mumbai Haematology Group at Seven Hills Hospital, Mumbai, from 3rd to 4th March 2012.

Mr. Vijay G Padwal, Asst. Library Information Officer

1. Attended a Seminar on "Competitive Intelligence", held at TMH, Parel, Mumbai on 25th May 2012.

Students:

1. Priti S. Nair presented a paper entitled "Mutations in F8 gene corresponding to B domain in relation to the clinical presentation" in the 35th Annual Conference of Mumbai Haematology Group at Seven Hills Hospital, Mumbai, from 3rd to 4th March 2012.
2. Patricia Pinto presented a paper entiled

"Standardization of an APTT-based FVIII Inhibitor Screening Assay" in the 35th Annual Conference of Mumbai Haematology Group at Seven Hills Hospital, Mumbai, from 3rd to 4th March 2012.

3. Tejasvita Gaikwad presented a paper entitled "Influence of CYP2C9 and VKORC1 polymorphisms on warfarin dose and other adverse effects." in the 35th Annual Conference of Mumbai Haematology Group at Seven Hills Hospital, Mumbai, from 3rd to 4th March 2012.
4. Snehal Mhatre presented a paper entitled "Atypical presentations and late onset of HLH with Perforin deficiency: Case reports" in the 35th Annual Conference of Mumbai Haematology Group at Seven Hills Hospital, Mumbai, from 3rd to 4th March 2012.
5. Suchitra Surude presented a paper entitled "Molecular Characterization of Leukocyte adhesion deficiency-I (LAD-I)" in the 1st National Primary Immunodeficiency Disorders Conference held at Chandigarh from 10-11th March 2012.
6. Patricia Pinto presented a poster entitled "standardization of an APTT-Based FVIII Inhibitor Screening Assay" in the 58th Annual Scientific and Standardization Committee Meeting of the International Society on Thrombosis and Haemostasis (ISTH) held at Liverpool, UK from 25th-30th June 2012.

Awards

Dr. K. Ghosh, Director:

1. Received Arun Kumar Banerjee Memorial Oration Award at Institute of Post Graduate Medical Education and Research, Kolkata on 16th January 2012.

Dr. Shrimati shetty, Scientist E:

1. Delivered the BGRC Founders Day Oration on 'Genetic diagnosis of haemophilia and other rare bleeding disorders' in the 35th Annual Conference of Mumbai Haematology Group Conference 2012, at Seven Hills Hospital, Mumbai from 3rd-4th March 2012.

Dr. Anita Nadkarni, Scientist D:

1. Awarded ICMR International fellowship for Bio-Medical Scientist (2011-2012) – senior grade and Visited Dr. E Kmiec Gene Targeting laboratory in Delaware state University, Delaware USA in Feb 2012.

Students:

1. Ms. Priyanka Kasatkar, was awarded with the 2nd Best poster award in the 35th Annual Conference of Mumbai Haematology Group held at Seven hills Hospital, Mumbai from 3rd to 4th March 2012 for the paper entitled "Polymorphic markers in VWF gene: Application in genetic diagnosis of Severe VWD families".
2. Dr Sushant Chavan, Research Associate awarded with the best oral presentation award for the paper entitled "Leukocyte Adhesion Deficiency I: To Delineate Molecular Defects in ITGβ2 gene and Utilize it in Prenatal Diagnosis" in the 35th Annual conference of the Mumbai Hematology Group held on 3rd - 4th march 2012.

Others

Dr. K. Ghosh, Director

1. Invited as external examiner to undertake MD (Medicine) examination at AIIMS, New Delhi from 14th-15th February 2012.
2. Invited as examiner to undertake Viva Voce examination at Pune University on 20th February 2012.
3. Invited to conduct MD exams at West Bengal University of Health Sciences, Kolkata from 9th-10th May 2012.
4. Dr. K. Vasantha, Scientist E superannuated on 31st August 2012 after completion of 35 years of service.
5. Dr. U Shankarkumar, Scientist D and Mr. M. Bane, Technician C left for heavenly abode after several years of service to the institute. The institute mourns their untimely death.

World Lupus Day celebration

Workshop on Molecular Analysis and Prenatal Diagnosis of Hemoglobinopathies

Lecture on Health and Happiness of Art of Living

Institutional Animal Ethics Committee meeting

Farewell to Dr K Vasantha, Scientist E

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