Manuscript Title-

Potential Role of Cathepsin K in Regulation of Micro Tumor Environment, Onset and Progression of Cancers in Human- A Review.

Running title-

Cathepsin K and Cancer in Human

Authors-

Ruwini Cooray[1*], Hasanka Madubashetha[1], P.D.S.U Wickramasinghe[2], Lakshan Warnakula[1, 4], Nimali De Silva[3]

Institutional affiliations-

1- Section of Genetics, Institute for Research and Development in Health and Social Care, Battaramulla, Sri Lanka.
2- Department of Chemistry, Faculty of Science, University of Colombo, Sri Lanka.
3- Department of Nanotechnology, Faculty of Technology, Wayamba University of Sri Lanka.
4- National Science Foundation, Sri Lanka.

***Corresponding author-**

Ms. Ruwini Cooray

Address for communication-

*Directly address the *corresponding author*

Ruwini Cooray,

Research Scientist and Lead,

Section of Genetics,

Institute for Research and Development in Health and Social Care,

No 393/3, Lily Avenue, off Robert Gunawardena Mawatha, Battaramulla,

Postal Code- 10120,

 doi:10.20944/preprints202003.0405.v1

Sri Lanka.

Telephone- Office- +94 112863084, Mobile- +94 777328324

Email- krncooray@live.co.uk

Conflicts of interest-

The authors have no conflicts of interest to declare on this work presented.

Funding-

Not applicable.

Ethical Considerations

Not applicable.

Acknowledgement

The authors acknowledge the Institute for Research and Development in Health and Social Care at Battaramulla, Sri Lanka for their support and guidance in various ways throughout the preparation of the manuscript, until been sent for publication. Acknowledgement is also extended to Professor Tsuyoshi Koide of the Mouse Genomics Resource Laboratory, National Institute of Genetics, Japan for resource support in writing the manuscript

 doi:10.20944/preprints202003.0405.v1

Abstract

The effect of proteolytic enzymes including Cathepsin K, a cysteine cathepsin, in onset and progression of cancers in human has been research intensive. Cathepsin K involves in many aspects and stages of cancers including apoptosis, cell proliferation, cancer immunology, inflammatory cell recruitment to tumors and aiding in the process of mobilization of normal healthy cells from their tissue compartments assisting in metastasis and angiogenesis. The objective of this review is to collect together and summarize and analyze the biochemical and physiological pathways of how cathepsin K is involved in onset and progression of cancers with more emphasis on breast and prostate cancers and cathepsin K regulated mechanisms underlying metastasis of such cancers to bones. Information for the review was gathered through published literature from global databases such as Google Scholar, PUBMED and NCBI on different studies on physiological interactions between enzymatic activity of cathepsin K with cancers and metastasis to bones. Analysis of published studies reveal that immunohistochemical studies of breast cancer cells indicate that they overexpress cathepsin K resulting in induction of aberrant mechanisms of cell signaling in breast cancers, creating a higher tendency for their metastasis to bones. Immunohistochemical, immunoprecipitation and fluorgenic assays of several studies done on the association of the same enzymatic activity on prostate cancers shows elevated levels of cathepsin K. Lesions derived from prostate cancer cell masses were observed to undergo increased bone formation and resorption levels. Such resorption levels cause secretion of biological factors promoting tumor expansion. In addition, studies indicate that Cathepsin K was observed to be a key component promoting higher bone resorption levels in patients suffering from cancer. Authors suggest that, to completely understand the association of cathepsin K on cancerous cells and their mechanism in metastasis, distributary patterns of cathepsin K in healthy human tissues needs to be extensively studied initially. It is also suggested that metastasis of breast and prostate cancers to bone could be terminated and overcome by successful production of efficient and precise inhibitory therapeutics targeting the enzymatic activity of Cathepsin K with minimum unintended adverse health effects.

Keywords- Cancer, Cathepsin K, Human, Metastasis, Physiology

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 March 2020 doi:10.20944/preprints202003.0405.v1

Introduction

Cancer has become a deadly pathological condition and a burden to lifestyles across the globe today with various attributes such as the extent of spread, localized effects, systemic effects and other acute and chronic disorders in human. Considering the latest statistical data available from the International Agency for Research on Cancer by 2018; through the World Health Organization reports that on a global platform the cancer burden is estimated to have risen to 18.1 million new cases and 9.6 million deaths in the year 2018. One in every 5 men and one in every 6 women worldwide develop and advance cancer during their lifetime while one in every 8 men and one in every 11 women die from cancer. At global level, the total number of people who are alive within 5 years of any cancer diagnosis, which is called the 5-year prevalence, is estimated to be 43.8 million. Reasons for the onset of cancers may include the exposure to carcinogenic substances, genetic predisposition or other implications of impaired or defective biochemical pathways that lead towards pathophysiological conditions in the body. The association of proteolytic enzymes with the onset and progression and cancers has been under intensive research since a long time and recently oncologists and oncobiologists, turning towards a more specific point of consideration and narrowing down, attempt to understand the association of lysosomal proteolytic enzymes and cancer in human. This review collects together, summarizes and analyzes the various published literature that show the association and influence of human cathepsin K, which is a lysosomal proteolytic enzyme, with the onset and progression of cancers in human including cancer cell behavior, effects and survival within a cancerous cellular environment.

Proteolytic enzymes involve in degradation of amino acids and proteinaceous compounds. In fact, it is also noted that the enzymatic activity of proteolytic enzymes could be either inhibited or stimulated by various other molecules in the cells and this fact accounts in better understanding the pathobiology of a wide variety of diseases. This is called the degradome (López-Otín and Overall, 2002). However, it should also be taken into consideration that this wide and complex interactions of proteolytic enzymes with inhibitors or substrates works in par with homeostasis in the context of normal physiology (Puente *et al.*, 2003). At pathophysiological conditions, this degradome undergo drastic changes. In instances where proteolytic enzymes do not function at

optimum levels, failing to exhibit their functional specificity and lead to cancer, the degradome changes to a cancer degradome (Doucet *et al.*, 2008).

The effect of a variety of proteolytic enzymes in the onset and the progression of cancers has been of great interest and an intensively research provoking area since many decades and continues to be the same (Akers *et al.*, 2013). In fact, the process of proteolytic enzymes is now proven to involve in many aspects of cancers including apoptosis, proliferation, immune response effect, inflammatory cell recruitment and most importantly aiding in the process of mobilization of normal healthy cells from their tissue compartments to assist in metastasis and interestingly in angiogenesis (Nakano, 2014).

Enzymatic structure and functions of proteases and their association with lysosomes have been studied in depth. It is noted that lysosomes are the storage compartments for a variety of acidic hydrolases, including all the cysteine cathepsins in human. In highly regulated biochemical processes including autophagy, phagocytosis and endocytosis, cathepsins involve in the intracellular degradation of substrates and regulate the processes of distribution and internalization of molecules in cells (Prasmickaite *et al.*, 2002) and recycling of different molecules taken up by cells from their immediate environment (Ad- and Alto, 1966). Studies reveal that in conditions of a cancer, such vital processes get altered drastically and lysosomes acquire a significant and an extensively higher proteolytic potential that could be considered highly relevant for cancer onset and progression in human (Aits and Jäättelä, 2013).

In human, 11 different cysteine cathepsins are identified which belong to the papain-like protease class of proteolytic enzymes in human, which is the largest and the most preeminent structurally and functionally characterized class of cysteine proteases (Brix *et al.*, 2008). The expression of cysteine cathepsins in human are highly different and are variable; both, with respect to the type of tissue and the developmental stage passed by the human (Turk, Stoka, Vasiljeva, Renko, Sun, Turk, *et al.*, 2012). The biosynthesis, activation and the regulation of cysteine cathepsins in human are complex processes that are highly regulated via a variety of post translational modifications (Turk, Stoka, Vasiljeva, Renko, Sun and Turk, 2012). The concept that cysteine cathepsins been active in reducing or rather slightly acidic environment in lysosomes is still under

investigations to be considered as an appropriate condition for their normal enzyme physiology (Saftig *et al.*, 1998). However, considering the relevance to cancer cell invasion, all cysteine cathepsins are found to be proteolytically active at higher pH conditions as well. It should emphasized that studies focused on cathepsin K and its recently recognized role in the progression of various types of cancer in human has been a novel area to explore in oncobiology (Quintanilla-Dieck *et al.*, 2009). In addition to the occurrence of cysteine cathepsins in cellular lysosomal compartments such as the cytoplasm and the nucleus, it should be noted that these enzymes are secreted either in the form of pro-peptides that are activated in situ or even in the active form (Kenig *et al.*, 2011).

Cathepsin K is encoded by a single-copy gene of approximately12.1 kb of genomic DNA on chromosome 1q21 with 8 exons and 7 introns. The primary function of cathepsin K in human is identified to be involved in the process if bone remodeling through a highly regulated pathway called ossification (Lecaille, Bro and Lalmanach, 2008). However, in addition, cathepsin K is also found to be distributed to some considerable extent in vascular smooth muscles, macrophages, white adipose tissue, synovial fibroblasts, chondrocytes and in the adult lung airway epithelium (Vidak *et al.*, 2019). It is also suggested that cathepsin K is involved in embryonic development, and that elevated levels of cathepsin K have also been identified in epithelial cells of different organs which are inclusive of the lungs, the gastrointestinal tract, bile ducts and kidneys and even in the heart valves (Fonović and Turk, 2014). In addition, it is also evident that genetic defects in the *CtsK* gene coding for the enzyme cathepsin K in human cause Pycnodysostosis which could be regarded as a specific form of osteoporosis, however, is a rare autosomal inherited disorder that is inherited through heredity causing abnormally hardened and denser bone, which makes bones more prone to easily undergo any form of brittle thus causing severe orthopedic disorders and complications (Hou *et al.*, 2003).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 March 2020 doi:10.20944/preprints202003.0405.v1

Research Methodology

An extensive search for published literature was done using global databases including NCBI; PUBMED and the Google Scholar. The keywords used were Cancer, Cathepsin K, Human, Metastasis and Physiology. Published studies from high impact journals were considered for building the review and making suggestions for future research avenues.

Results and Discussion

According to published literature in global databases such as the NCBI; PUBMED and the Google Scholar, cysteine cathepsins have been studied in particular in the process of cancer such as invasion and metastasis, tumor growth and attenuated apoptosis, angiogenesis and the different types of responses cancerous cells to inflammatory mechanisms (Moore, 2013). In addition, in accordance with the results derived from past research on analyzing the expression of cathepsin K in different cancer types in human, it is proposed that cathepsin K functions in a rapid and invasive form facilitating the growth of cancer in the form of sarcomas, carcinomas, melanomas and central nervous system tumors in a similar way and a rate as it involves in bone tissue degradation, which is its most basic and primary physiological function in human as illustrated above(Cardoso, Maciel and de Paula, 2014) . In particular, the heavily and intensively studied sarcomas include the ones that occur in the bones, kidney and the uterus in female where abnormal elevation in the Cathepsin K levels cause extensive osteolysis, further progression of the tumor along the tissue concerned(in uterine tissue associated cancers) and irregular adipocyte differentiation. Carcinomas here include the ones that occur in the breast in female, lungs, prostate in male, skin, thyroid, cervix in female, kidneys, stomach, head and the neck (Taxel, Choksi and Van Poznak, 2012). The physiological mechanism associated here is that abnormal degradation of the extra cellular matrix degradation (Everts *et al.*, 2003) and angiogenesis is promoted and an elevated degree of invasiveness of carcinoma cells through paracrine interactions are caused by abnormal elevations in cathepsin K trigger the progression of cancers and Melanomas on the skin, are been reported , where abnormal and wrong regulation of cathepsin K distribution can alter a variety of biochemical pathways affecting the normal physiology of cells in the skin tissue (Boonen *et al.*, 2012). The effect of

cathepsin K on the central nervous system tumors in the brain are also been reported to have been studied.

The effect of cathepsin K in causing cancers is well supported by the tumor microenvironment at cellular level due to its potential to support carcinogenesis, angiogenesis and the ability to regulate immunomodulatory functions (Boutté *et al.*, 2011). Studies suggest that basically the stromal cells in the tissues of the body in a particular organ attract to the tumor as the major host defense response to neoplasm, and may transform into tumor-supporting cells and these processes may lead to propagation of the tumor throughout the body more conveniently (Raposo *et al.*, 2015). Stromal expression of cathepsin K has been highly observed in tumor-associated fibroblasts and macrophages of invasive adeno-carcinomas and basal cell carcinoma conditions as per reported studies (Ishida, Kojima and Okabe, 2013) .

In addition, the precise biochemical pathways by which cathepsin K expression is regulated in the process of progression of the tumors are not known to a greater extent (Kleer *et al.*, 2008). In published gene expression studies, a variety of transcription factors, such as SP1, AP1, AP3, H-APF-1,PU.1, ETS-1, PEA3, MiTF and TFE3 have been reported to be identified in the promoter region of the gene coding for the cathepsin K enzyme, in particular, the *CtsK* gene, to have some effect in the progression of cancers in human along with cell signaling mechanisms (Hira *et al.*, 2015). In a more specific context, in breast carcinomas, it is suggested that the activity of cathepsin K to be regulated by CD44 receptor signaling and by coronin 3 in gastric cancer metastasis (Ren *et al.*, 2012).

Studies also report that the expression levels of the enzyme cathepsin K could also be used as a biomarker to facilitate the identification of any presumptive sign leading to the prognosis of certain cancer in human (Kleer *et al.*, 2008). The expression of cathepsin K in lung carcinoma is associated with reduced recurrence and can be used as diagnostic bio marker to distinguish between non-invasive and invasive tumors (Tumminello *et al.*, 2008). There is also sufficient literature to support the fact that the expression of cathepsin K has been shown to be an effective bio marker to be used even as a differential marker for the precise and accurate diagnosis of tumors that exhibit the same phenotypic features such as rate of cell proliferation, genomic instability, cell

immortality and the distinct ability to interrupt and cause changes to both local and distant tissues (Cordes *et al.*, 2009). Moreover, a greater diagnostic value of cathepsin K was shown to be proved in the detection of thyroid carcinomas. In addition, in an investigation to differentiate between prostate and breast cancers, it has also been noted that Cathepsin K did not appear to be a suitable serum bio marker to detect any form of metastatic spread or occurrence of breast carcinoma (Novinec and Lenarčic, 2013) to different types of bones in the body, governed by the fact the levels of cathepsin K levels in the serum of breast and prostate cancer patients and a control group did not differ statistically (Suoranta *et al.*, 2012).

Accordingly, recent studies and their outcome suggests that cathepsin K is overexpressed in many different types of cancers in human (Brömme and Okamoto, 1995). Within highly metabolically active cancer cells of the tumor mass, cathepsin K could directly or indirectly affect the cell signaling pathways, neural coordination and involve in abnormal and unnecessary degradation of extracellular matrix proteins, which are metabolic processes that has a particular and a strong consideration in bone metastases (Lindeman *et al.*, 2004). Most importantly, cathepsin K has also been reported to express in conditions of glioma, which are different types of tumors that occur in the brain and in the spinal cord, regulating the mobilization ability of a group of cells like cancer stem cells contributing towards the growth, development (Ahlemann *et al.*, 2006) and the metastasis of cancer cells. However, the study suggested that the importance of efficient inhibitors for cathepsin K would be the most effective solution, that is, to treat and prevent the metastasis of glioma cancerous conditions to the bone and further progression of the cancer, causing devastating and adverse health effects that might require chronic medication (Verbovšek *et al.*, 2014). The study also suggested that the effect of cathepsin K inhibitors on suppressing the physiology and the metabolism of cancers in human has to be given more and immediate clinical attention, that would in turn enable the pharmaceutical industry to create novel and efficient inhibitors suppressing the activity of cathepsin K related, stimulated or progressed or triggered cancers in human (Mukherjee and Chattopadhyay, 2016).

Considering the global status of cancer patients, it could be suggested that it is high time that clinical trials move from laboratory animal to human with this regard on immediate consideration and to bring such treatment practices in reality.

As such, having analyzed the scientific background above, it is understood that abnormal genetic and biochemical changes in cathepsin K could also lead to a variety of cancers in human in addition to other disorders such as osteoporosis or PKND (Lecaille, Brömme and Lalmanach, 2008). It could also be suggested, that distribution of cathepsin K in tissues of the body and their specific effect on different tissue type needs to be better understood in the most initial place, in attempting to interpret the oncology related to cathepsin K (Brömme and Lecaille, 2009).

Having clearly illustrated the biochemical and the physiological concerns of the association of the enzymatic activity of Cathepsin K on cancers in human, hereon, the focus of the review is more specific on relating published literature that show evidence for various studies that have analyzed and related the association of cathepsin K and specific types of cancers in human. This analysis further supports and governs the major objective of this piece of writing, which is to collect together, summarize and analyze various recently published literature that show the association and influence of human cathepsin K with the onset and progression of cancers relating to their pathophysiology and the us of concepts in genetics and molecular biology has supported such studies. In addition, this review shall provide a key to investigate and mitigate on the different approaches in molecular biology that could be taken or rather applied in bridging any research gap identified, with pathobiological concerns on this diseased or rather the abnormal biochemical trigger on cancers by cathepsin K.

The effect of cathepsin K on breast cancer metastasis to bone tissues in human has been studied into depth and researches have shown keen interest into thorough and comprehensive studies with regard to this since the high prevalence of breast cancer in women and the resulting orthopedic disorders associated therewith (Gay, Gay and Koopman, 1993). A variety of immunohistochemical assays are reported to be conducted on breast cancer cells and analysis of the results have validated the fact that breast cancer cells overexpress Cathepsin K. During the first reported study that was done to detect the presence of cathepsin K in breast cancer cells, cathepsin K was localized in primary and metastatic breast tumors using in situ hybridization and immunolocalization. In addition, using techniques in molecular biology, the gene encoding for cathepsin K was amplified by reverse transcription polymerase chain reaction (RT-PCR) in the breast cancer cell lines that were prepared in primary and

metastatic or rather secondary stage breast tumors, to reflect the fact that the gene for this particular protease is expressed in these tissues. The study was also capable of presenting the fact that none of the breast epithelial cell lines derived from noncancerous breast tissue expressed the enzyme cathepsin K which was validated by RT-PCR followed by a secondary Southern blot analysis. During this study, most importantly, the mechanism of how cathepsin K could involve in breast cancer metastasis was initiated to be studied. The study suggested that since cathepsin K is in active state under acidic conditions and also have shown to be capable of degrading extracellular matrix proteins, this provides a possible mechanism by which breast cancer cells can metastasize to secondary sites, especially the bones. In fact, bones, which have been identified as a frequent and a common site for breast tumor metastasis in women, which is rich in osteonectin and collagen, are both effective substrates for the expression of the degradative action of cathepsin K. In addition, the study was also able to emphasis the fact that in the identification of cathepsin K in primary breast cancer cells and in cells that are metastatic to the bone cathepsin K may play a highly effective and a noteworthy contributing role in the process of tumor cell invasion. Most importantly, the study was able to present through RT PCR analysis that MCF-7 cells express cathepsin K, providing an indicative suggestion that breast tumor cells may be able to directly resorb bone via the release of this proteolytic enzyme (Littlewood-evans *et al.*, 1997). Therefore, on obvious grounds it is understood that this study has used a variety of tools in molecular biology and further secondary metastatic expression studies in tissues that are suggestions of the study could also be supported by the application of novel techniques in molecular biology more effectively (Bartek *et al.*, 1991).

An advanced study conducted recently based on the fact that all cathepsin proteases including cathepsin K, have many cell-specific functions within the tumor microenvironment that facilitate the promotion of tumor growth and invasion as illustrated above, the activity and expression of cysteine cathepsins in a mouse model of breast cancer metastasis (Sevenich *et al.*, 2011) to bone was studied intensively to investigate for how conclusions based on the study could bring up innovative and novel research avenues with relation to human. The results of the study was able to reveal the fact that cathepsin K is strongly upregulated during the transition from

MDSCs/macrophages to osteoclasts while other cathepsins such as X, L, and B expression (Vasiljeva *et al.*, 2006) and their corresponding activities are downregulated. The study also indicates that delayed onset of the effect of cathepsin inhibitors and ineffective and deteriorated activity on precursor proliferation could suggest that this is due to a fusion-mediated effect. The study also indicates that increased osteoclast activity in the bone tumor microenvironment has been associated with enhanced metastasis and bone degradation, and osteoclasts might be performing some active physiological activity in promoting extensive outgrowth from tumor cell dormancy. The study suggests and validates that targeting specific cathepsin activity inhibition including cathepsin K could inhibit breast cancer metastasis. The study also supports the a potential bone metastasis suppressive function of MDSC-derived cathepsins (Danilin *et al.*, 2012) which need extensive studies and validation.(Edgington-mitchell *et al.*, no date).

Another study which effectively emphasized on the fact that cathepsin K was expressed not only by osteoclasts but also by breast cancer cells that metastasize to bone through immunohistochemical studies, strongly upstretched the possibility that cathepsin K could be a therapeutic target for the treatment of bone metastases of breast cancer cells. This conclusion was based on intratibial injective administration of cathepsin K expressing human BT474 breast cancer cells to mice. The tumor-bearing mice were subjected to be treated with a particular type of clinical dosing regimen of cathepsin K inhibitor, produced osteolytic lesions that were 79% smaller than those of tumor-bearing mice treated with the vehicle or rather the same non-cancerous breast cells. Simultaneous studies were also done in analyzing the effect of the same CKI in mouse models in which the *in vitro* inoculation of human B02 breast cancer cells expressing cathepsin K leads to bone metastasis formation. The study concluded the fact that CKI may render the bone a less favorable microenvironment for tumor growth by inhibiting bone resorption with a lowered skeletal tumor burden which is a noteworthy and a timely outcome that could be used to understand the background of therapeutics that are required to inhibit the activity of cathepsin K that might differentially and anomaly lead towards causing breast cancer metastasis cancer (Gall *et al.*, 2007). However, an important fact that needs to highly subjected to constructive criticism on grounds of molecular oncological research is that unlike benign cancers that have a wide variety of

effective treatment protocols to completely eliminate the disorder effectively as an outcome of intensive research (Mundy, 2002), treatment options to combat malignant cancers are relatively lower in terms of option. Therefore, such research that is related to understanding in depth biochemistry and physiology is highly important to be brought to an area of high activity in combating and controlling such malignant cancers and such research needs to be better promoted (Sheridan *et al.*, 2006).

An imperative physiological study on a molecular platform level revealed the metabolic potential and the capability of cathepsin K to induce platelet dysfunction and the effect of cathepsin K in cell signaling in breast cancer. It was previously understood that the breast cancers in human encompasses different tumor sub groups that are clinically and molecularly distinct making momentous changes in cellular, histological (Mohamed and Sloane, 2006) and biological features and characteristics demonstrating a variety of different clinical expressive features including their response to different cathepsin K inhibitors (Lecaille, Bro and Lalmanach, 2008). This study in particular correlated the epithelial mesenchymal like transition breast cancer cells in human and the resulting cathepsin K secretion with activation and aggregation of the platelets. Cathepsin K is being highly regulated in cancer cells that subject the cellular extracellular matrix to effective proteolytic degradation leading towards invasiveness. Even though a variety of clinical studies have been able to clearly and effectively show that proteolytically activated receptors (PARs) are activated by different proteases themselves, the direct interaction of cysteine cathepsins with PARs is not understood and comprehended in total with relation to physiology and biochemistry including the related metabolic pathways. This study reports to have taken into consideration the fact that in human platelets, PAR-1 and PAR−4 are highly expressed, but PAR-3 shows low expression and unclear functions. The study concluded that there is a high probability that cathepsin K might activate PAR-3 and PAR-4 mirroring the activity of the enzyme α-thrombin on these substrates. Cathepsin K also induces platelet aggregation in a dose-dependent manner and triggers Src and p38 phosphorylation and calcium ion influx from the contents of platelets inducing platelet dysfunction, which could facilitate the interaction with breast tumor cells involving a successive series of signaling events linked to cathepsin K induced platelet activation and aggregation when co-cultured with breast cancer cells from patients with luminal B type carcinoma (Andrade *et al.*, 2016). Hence

it is concluded that cathepsin K induces platelet dysfunction which hinders healthy physiology and affects signaling of breast cancer metastasis. However, with the advent and advancements in enzyme functional studies of proteases along with protein and genetic engineering efficient and stringent enzyme inhibitors would evolve to avoid such metabolic disorders (Ludeman *et al.*, 2005). Such studies and application of their outcomes on clinical trials should be considered important and timely, that would cause improvements in the health of oncological patients on a global platform.

The above information successfully grounds to the fact that breast cancers frequently metastasizes themselves to the skeletal system, which hinders and occasionally deter the normal metabolic processes that are associated in bone remodeling in human while also inducing and stimulation the process if abnormal and unnecessary bone degradation (Ell *et al.*, 2013). Recently, by taking into consideration many studies done with regard to this by Vargas and colleagues reported through a review, the different molecular mechanisms through which osteoclasts and breast cancer cells function in conjunction with each other resulting in a trigger to the occurrence of osteolytic bone metastasis. It was reported that in an incident in which the metastatic cancerous cells colonize the bone tissues they interact with the osteoclasts and the osteoblasts and collectively induce bone degradation in addition to their activity alone, making the process more effective. As a response, many bone derived growth factors and the calcium components and the ions released from the resorbed bone tissues, stimulate the formation of skeletal tumor growth at abnormally devastating rates. In biochemistry, this concept in detail is referred to as the vicious cycle. In addition, a variety of other molecules that are produced by the breast cancer cells in specific including interleukins (IL-8, IL-11), M-CSF and TNFα are involved in direct stimulation of the activity of the osteoclastic activity to anomaly higher rates which cause bone degradation and the resulting metabolites by degradation are the key components in the formation of bone tumors as illustrated earlier. In addition, osteoclasts also involve in the secretion of exosomes containing miRNAs such as miR-378 that has the metabolic potential to promote extensive growth of tumors, angiogenesis and tumor cell survival through the repression of tumor suppressors such as SuFu and Fus-1 which is an important and a noteworthy consequence. In particular, it also needs to be taken into consideration that the bone itself is a massive pool of a variety of growth factors and calcium components

can broadly stimulate the growth of tumor cells. Activated TGFβ stimulates and the PTHr expression by tumor cells which stimulate osteoclast mediated bone resorption resulting in the formation of a variety of clearly undefined tumors. Members of the protein family called bone morphogenic protein family which includes the proteins IGFs, PDGF and BMP are released from the bone matrix and they enhance tumor cell proliferation. A highly important fact of extreme consideration is that calcium acts on tumor cells that express the calcium sensing receptor (CASR) by creating a prolonged and a highly supportive environment for the extensive survival of tumor cells in the body. In fact, the enzymatic role exhibited by the enzyme cathepsin K determines the extent of the effect of the factors stressed above since this is the key enzyme that is involved in regulating the physiology of bones (Takayanagi, 2018). However, in a gross concern it has to be profoundly taken into consideration that tumor cells produce a considerable proportion of soluble factors that promote and encourage bone degradation. On the other perspective in certain cases it has also been reported that tumor cells may produce factors that inhibit osteoclast activity such as endothelin-1 and OPG, leading to the formation of osteoblastic or a variety of mixed lesions that needs to properly understood and better comprehended via further research. In effort to establish this relationship with the influence of cathepsin K produced by the tumor cells, it was reported to promote and trigger tumor cell invasiveness and directly and actively contribute to bone degradation (Le, Vargas and Clézardin, 2016). Accordingly, it is understood that underlying mechanisms of bone metastasis is complex and involves a very high degree of cooperative interactions among the metastasizing tumor cells, osteoblasts, osteoclasts and the associated mineralized bone matrix (Edwards and Mundy, 2011). Techniques and principles in molecular biology should be recruited in filling up such gaps unidentified in attempting to comprehend the complete pathophysiology in such concerns. For instance, it could be suggested that ChIP sequencing be recruited in identifying the protein-DNA or protein-RNA interactions that cathepsin K might have with cell signaling molecules and such pathways in metastasis of deadly cancers to bones in human.

Further, intensive research also related the relationship between the occurrence and the progression of breast and prostate cancers with cathepsin K. However, to date it is evident that bone metastasis that are associated with later stage prostate or breast

cancer (Fradet *et al.*, 2011) has a keen potential to develop skeletal complications which includes bone pain, hypercalcemia, pathologic fracture, compression of the spinal cord, and instability in the spinal cord instability (Futakuchi, Fukamachi and Suzui, 2015).

The physiological process by which prostate cancer invasion and metastasis occur are highly facilitated by a sequential group of proteolytic enzymes that include multiple proteases out of which cysteine proteases including cathepsin K, been highly dominant (Hol, Wilhelmsen and Haraldsen, 2010). Earlier it was elaborated on the fact that prostate cancers and breast cancers, with a high degree of affinity and feasibility, conveniently metastasize to the bone tissues (Khodavirdi *et al.*, 2006). In fact, studies reveal that the expression levels of the enzyme cathepsin K is greater in prostate cancers bone sites compared to primary tumor and normal prostate tissues which itself is a phenomenon that needs vital attention to be investigated (Schmit *et al.*, 2012) on since the exact reasons causing this abnormal elevation was neither clearly understood during the particular study of reference or nor to date.

The most commonly seen malignant cancer in men are prostate cancers and is reported to be highly associated with bone metastasis, which is the closest and the relatable reason that cause the morbidity of prostate cancers and the lesions that are derived from such cancerous cellular masses (Tamada *et al.*, 2001). These lesions are also observed to undergo increased bone formation and resorption levels. Such resorption levels cause the secretion of different biological factors that could promote the tumor growth and development (Charhon SA, Chapuy MC, Delvin EE, Valentin-Opran A, Edouard CM, 1983). From the time of inception of analyzing the effect of cathepsin K with prostate cancers, as per the published literature in several renowned global databases, the use of several molecular biological techniques such as the RT-PCR and DNA sequencing is reported to have been used extensively. Therefore, it could also be suggested that the establishment, expansion and development of studies and facilities that could link and correlate molecular biology and oncology is vital for the output of precise and acute conclusions on oncological diseases and their insights, resulting in timely and treasured outcomes that improve the quality of life and healthy well been in mankind (Fonager *et al.*, 2017). However, it should be noted that tangible outcome is been brought out through the study that could contribute actively in onco-medicine in the resent day; it has to be in reality and effective.

In an initial study to investigate the effect of Cathepsin K on prostate cancers, the expression levels of cathepsin K in cell lines derived from patients diagnosed with prostate cancers were studied. Further and intensive examinations for the expression of the native form of cathepsin K was done using immunohistochemical studies, immunoprecipitation followed by western blotting and a variety of confirmative fluorogenic assays and was compared with healthy tissues obtained from the prostate and patients suffering from primary or the very initial stages of prostate cancer (Chikatsu *et al.*, 2000), it was observed that the levels of cathepsin K in secondary cancers were abnormally higher compared to the primary ones of the same type and that the healthy prostate tissues did not express cathepsin K at all. Therefore, the study suggested that the presence and expression of cathepsin K in patients suffering from later stage prostate cancers are actively involved in contributing to the invasive nature and in rendering the invasive potential to prostate cancers in men (True *et al.*, 2003).

A recent study was done on investigating the functional role of cathepsin K during the growth stage and the developmental stage of prostate cancer in the murine bone. A molecular biological approach was used in this perspective of the study by initially validating the cathepsin K mRNA expression by RT-PCR, validating the native protein expression by immunoblotting in prostate cancer LNCaP, C4-2B, and PC3 cells as well as in the corresponding tissues by final confirmatory steps which involved in measuring the protein production using an ELISA assay. The effect of siRNA and cathepsin K inhibitors were compared in relation to prostate cancer cell invasion. As outcomes of the study, it was confirmed that cathepsin K expression in prostate cancer LNCaP, C4-2B, and PC3 cells as well as in prostate cancer tissues were dominant. In addition, according to the results of the inhibitory effects of a selective cathepsin K inhibitor on prostate cancer cell invasion, it has been observed that the cathepsin K inhibitor dose dependently inhibited prostate cancer conditioned media-induced bone resorption. It was also suggested that the inhibitory effects of the cathepsin K inhibitor were enhanced in combination with zoledronic acid (Gandaglia *et al.*, 2015). The study concluded with emphasis on the fact that efficient inhibitors that can inhibit the enzymatic activity of cathepsin K inhibitors (Bonnet *et al.*, 2017) may prevent the establishment and progression of prostate cancers in bone, resulting in highlighting a novel but yet a standardized and a reliable, precise therapeutic in order to be used to

treat prostate cancers, eventually by facilitating complete recovery from even at the secondary stage of the prostate cancer (Liang *et al.*, 2019).

In addition, a study that was conducted using mice models in investigating the effect of the degree of bone marrow adiposity in causing obesity and other associated pathological disorders such as the increased marrow fat content, abnormal bone degradation and the occurrence of prostate tumors was able to demonstrate that the expression of the native form of the protein cathepsin K (Raggatt and Partridge, 2010) in osteoclasts grown in media condoned by marrow adipocytes, have more protein expression of both the 37 kDa inactive pro-cathepsin K and 28 kDa mature or rather the active form of the enzyme. This suggested that the adipocyte-derived factors increase the metabolic process of osteoclastogenesis and the proteolytic activity of osteoclast derived cathepsin K in *in vitro* conditions. This suggests that different skeletal related events such as bone fractures, spinal cord compression, pain in the bones and condition such as hypercalcemia (Rosen and Bouxsein, 2006) are involved in the progression and invasiveness of prostate cancers and studies related to understanding such physiological mechanisms in the human body has to better studied and comprehended, it could also be suggested that the use of sophisticated molecular biological techniques be applied to such concerns, thereby, providing better understanding of the molecular interactions that are involved in such physiological manifestations in the body (Hardaway, Herroon and Rajagurubandara, 2015).

A study was also reported to have studied the physiology, pathophysiology, enzyme mechanism and the expression levels in the involvement of the enzyme cathepsin K in oral tongue squamous cell carcinoma and HSC-3 tongue carcinoma cells and two oral keratinocyte cell lines, oral carcinoma associated fibroblasts (CAFs) and primary gingival fibroblasts (GF) were treated with IL-1α, pertaining to the broad intention of investigating the effect of interleukin (IL)-1α on the invasion of oral tongue squamous cell carcinoma (Yan *et al.*, 2011). The investigation was based on analyzing the expression of Cathepsin K in tissue samples with oral tongue squamous cell carcinoma with immunostaining and other related histochemical immunological techniques. Accordingly, the cathepsin K expression was measured and interpreted using PCR and ELISA. During the interpretation of the results, it was demonstrated that the enzyme cathepsin K was found in the vesicles of the cells affected with oral tongue squamous

cell carcinoma, very close to the cell membrane, yet within the exosomes. In addition, it was also demonstrated that cathepsin K was expressed at the basal level in both the epithelial cells and fibroblasts, basal IL-1α levels were higher in epithelial cells compared with GFs and CAFs (Ganly, Patel and Shah, 2012). This suggested that the cathepsin K expression was slightly induced by IL-1α in all cell lines, but it did not affect in the HSC-3 cancerous cell invasiveness. In conclusion, the study emphasized on the fact that IL-1α induces cathepsin K in epithelial and mesenchymal cell lines under *in vitro* conditions. In addition, it was also seen that in OTSCC cells, subcellular compartments, even though not secreted, has the potential of quickly mobilizing the enzyme cathepsin K. This led to open up further study avenues to investigate whether this mechanism of mobilization is a defensive action by cancer cells in human to facilitate in their survival and invasion throughout processes involved in tumor progression. Therefore, on a strong scientific platform, this particular concern that was raised through this study needs to be addressed to better understand the role of cathepsin K in the progression of cancers and their specific behavior within a tumor micro environment (Bitu *et al.*, 2016).

Accordingly, it is clearly understood that cathepsins in whole are highly associated in particular with the progression, invasiveness and metastasis of cancers in human. In addition, they are also found to overexpress themselves in a variety of cancers. Metastatic lung cancer is reported to be the leading cause of cancer-associated mortality in a global perspective and therefore the immediate necessity for the identification and comprehension of the different concepts in metabolism, physiology and the pathophysiology causing lung cancers be understood. However, recently the necessity for understanding the genetic background of lung cancers has also come into the arena and is been addressed on grounds of the related molecular physiology and even other downstream applications such as therapeutics that can avoid lung cancer cell invasion (Siegel *et al.*, 2014). In support to this concern, a recent study that was conducted on mice models reporting of an *in vivo* gain-of-function genetic screen to identify driver genes of lung cancer metastasis. In the study reported here, *TMEM106B* was identified as an important driver gene involved in causing lung cancer metastasis. It is reported that the gene *TMEM106B* is capable of directly involving in the regulation of the expression of cathepsin K in lung cancers. As illustrated previously, since cathepsin K

also involves in metastasis of cancers and the progression of tumors, this study further validated the fact that the influence of cathepsin K in ling cancer and their metastasis also has a similar effect. The study was also capable of successfully demonstrating the fact that abnormal and irregular expression of the gene *TMEM106B*, which encodes a transmembrane protein in human, could pointedly promote the synthesis of significantly enlarged vesicular lysosomes that are highly composed of abnormally elevated levels of a variety of active cathepsins. The study also showcased the fact that *TMEM106B* induced lysosomes are capable of undergoing extensive metabolism in terms of calcium dependent exocytosis resulting in the production and releasing of active lysosomal cathepsins necessary for *TMEM106B* dependent cancer cell invasion and metastasis, yet in *in vivo* conditions. However, these observations and conclusions have to better understood in human models to better relate and understand the underlying mechanisms of cancer invasiveness and progression and the related pathophysiological concerns (Kolter and Sandhoff, 2010). However, this particular studied concluded the fact that the activity of the *TMEM106B* gene to cause cancers could be inhibited by successfully inhibiting the activity of cathepsins with the use of pharmacologically efficient therapeutics as cathepsin inhibitors. The study was significant in terms of which could be effective therapeutically prevented by pharmacological inhibition of cathepsins that promote the tumor progression activity of cathepsins and in particular the activity of cathepsin K (Kundu *et al.*, 2018) and (Breznik *et al.*, 2019).

Within the scope of the different human cancers that are observed across the globe today, gastric cancers too show a significant prevalence (Gupta and Massagué, 2006). A variety of gastric oncological studies have been conducted so far attempting to understand the related pathophysiology associated with their occurrence including acute and chronic health effects to human (Valastyan and Weinberg, 2011). However, from published literature, it is observed that a keen attention and noteworthy attempts are taken to understand the molecular perspectives of gastric cancers. In evidence to this concern, a recent study depicts the association of different biomolecules, in particular proteins, in different gastric cancer metastases and the related metastatic behavior and most importantly showing the effect of cathepsin K on gastric cancer metastases. The protein of concern here is Coronin, which was highlighted during the

introduction of this review as well, which are a highly evolutionary class of proteins with conserved domains which are involved in the metabolism and regulatory processes of the cytoskeletal behavior, especially of the actin components in it. However, the coronin 3 type is reported to have correlation in processes of cancer cell metastases. This fact is confirmed by previous literature that showed the elicit/inhibition of coronin 3 inhibiting the metabolic pathways related to cancer cell proliferation and invasiveness of glioblastoma cells, in turn inhibiting cell proliferation and invasion. However, in this study the migration, invasiveness and metastatic abilities of gastric cancer cells such as SGC7901, AGS, KATOIII, MKN-45, and MKN-28 treated with fluctuation levels of coronin 3 was studied and the mechanism of coronin 3 in cancer metastasis was clearly illustrated. In analyzing the results, it was found out that the expression of coronin 3 was higher in the highly metastatic sub-cell line MKN28-M. Within the study, the human tumor metastasis PCR array which was used to screen the metastasis associated genes identified by the down regulation of coronin 3, and the results obtained therein suggested that, after the knockdown of coronin 3, the tumor cell migration, progression and invasion were inhibited as a direct result of the reduced expression of MMP-9 and cathepsin K within the tumor. This suggests that it is due to a co regulatory mechanism of cathepsin K that is functional within the gastric cancerous cells, that their invasiveness and tumor progression is facilitated. Therefore, it could be concluded that with successful cessation or rather inhibition of the functional role of cathepsin K in the activity of coronin 3 and/or cancerous gastric cells may avoid the metastases of gastric cancers causing complex oncological disorders (Ren *et al.*, 2012).

Previously on the review, clear and profound illustrations in support to the fact that interaction of the tumor and is micro environment and relating such biochemical features are of high importance in cancer metastasis were highlighted and justified. Another study conducted and published recently also provides evidence in support to the association of cathepsin K with the progression of cancer and cancer invasiveness of gastric cancer reflecting the importance of the tumor micro environment (De Rosa *et al.*, 2015). Having considered the intestinal micro environment in human, the microbial population dwelling within it is clearly involved in the progression of a variety of colorectal cancers. A clear and a comprehensive explanation in relation to the underlying pathophysiology of how this takes place is not clearly understood in human.

However, this study was able to clearly identify and devote to the scientific table that cathepsin K is highly involved in the process of colorectal cancer metastasis by acting as a vital mediator between imbalance intestinal microbiota and colorectal cancer metastasis which could be classed as novel-science interest provoking. The normal human colon epithelial cell line FHC, NCM460, and colorectal cancer cell lines including SW480, SW620, HCT116, LS174T, HT29, and LOVO were used in the study and were manipulate in mice models for further intervention. As results of the study bigger and enlarged tumors along with more liver metastatic foci were detected in the cells that were supplemented with an *E.coli* bacterial population. In detail, cathepsin K also showed the potential to stimulate the process of secretion of the cytokines by M2TAMs including IL10 and IL17, which, in response to this mechanism, enhanced the invasion and metastasis degrees of colorectal cancer cells through NFκB pathway. Hence it was presumed that cathepsin K contributes to metastasis of colorectal cancer(Routy *et al.*, 2018). Eventually when the cells were treated in such a way by creating a knock down to avoid the production of cathepsin K or by the provision of the enzyme inhibitor Odanacatib, it was seen that the migration, mortality and the degree of the colorectal cancer metastases reduced drastically. Therefore, it could be suggested that the progression of tumors associated with colorectal cancers and their metastasis could be successfully suppressed by inhibiting the enzymatic activity of cathepsin K within the colorectal cancerous cells and tissues. Studies of this nature emphasis that internal physiological mechanisms have to better studied and understood, which results in the outcome of the most effective and the timely therapeutic that could be used in reliable disease treatment (Li *et al.*, 2019). This study also might open a pathway for the investigation of potential microbial products or their physiology that could inhibit some active componental physiological aspect of cathepsin K associated tumor metastasis in human.

A study that was conducted by Christensen and colleagues reveled that Matrix-metalloproteinases 9 (MMP-9), which is an enzyme involved in the extracellular matrix degradation (Xue *et al.*, 2014), that has shown evidence in the onset and regulation of cancers in previous studies, to have a functional association with cathepsin K in the physiological regulation of cancers (Everts *et al.*, 1992). The results of the study indicated that cathepsin K can cleave and activate MMP-9, especially in acidic

environments in physiological conditions in human such as in tumors and the process of bone resorption. Through the study that authors suggested that novel pharmacological targets and physiological interpretations in terms of medication could be brought forward, to inhibit the activity of MMP-9, by regulating the activity of Cathepsin K in the extracellular space. Accordingly, the progression of cancers could be avoided. This study also indicated that careful studies of the protein signaling pathways in human could reflect an extensive background related to the biochemistry of cancers which would make a better understanding on illuminating the different options of treatment that could be made available to cure cancers, the least been to avoid further progression of the cancer on a systemic outlook of the body making complex metabolical pathways that would later be difficult to cure (Christensen and Shastri, 2015).

A study done on investigating the clinicopathological implications of cathepsin K gene expression in oral squamous cell carcinoma which constituents the most common malignancy of the head and neck region (Ahmedin Jemal, DVM *et al.*, 1999). The study was able to identify the fact that an abnormal elevation in the gene and protein expression; CtsK and cathepsin K were strongly associated to lymph node metastasis. This study concludes and strongly emphasizes on the fact that up regulation of cathepsin K gene expression is a key reason with very high incidence for the occurrence of lymphatic node metastasis and poor survival in oral squamous cell carcinoma and also highlights that the CtsK gene could be used as a predictive biomarker for oral squamous cell carcinoma detection and analysis of the degree of the carcinoma (Leusink *et al.*, 2018). This recent study is therefore a suggestive indication for uplifting recruitment strategies of molecular disease diagnostics; molecular biological techniques especially in oncological medicine for disease detection and validation.

Recent studies have also been able to show that the overexpression, abnormal localization and aberrant physiological and metabolical functions of certain cysteine cathepsin including cathepsin K in various cancers of the body (Westhoff *et al.*, 2013). A significant level of overexpression was studied to be seen in glioblastoma conditions that occur in cancer stem cells. Moreover, the study also stressed and highlighted that cathepsin K is also involved in the activation and the inactivation processes of different cytokines that play an important role in the cell signaling pathways that has a strong effect on angiogenesis. However, it should also be considered that recently, cathepsin K

was also identified as one of the highest differentially expressed peptidases in glioblastoma tissue and cell lines when it was compared to its normal equivalents (Pišlar, Jewett and Kos, 2018).

Conclusions and Suggestions

In conclusion it could be stated that Cathepsin K is highly associated and exerts an important and a momentous effect on the occurrence, onset and progression of cancers in human with more affinity towards triggering the process of cancers. The authors have taken a worthy attempt to relate conditions with regard to the bone metastatic processes of different cancers including prostate cancers and breast cancers etc. Through the review the authors suggest that the expression patterns of cathepsin K in human body tissues have to be subjected to more intensive research and completely understood in order to better comprehend the biochemistry, metabolism and the physiology that is associated with overexpression nature of cathepsin K. It is within the mere interest of the authors to make an impressive suggestion which is that the different cell signaling pathways of proteinases including cysteine cathepsins have to be better understood including their functions and regulation The authors also suggest that novel, competent and efficient inhibitors in order to suppress the enzymatic activity of cathepsin K has to be identified leading to the direct, efficient and optimum production of therapeutics and inhibitors that would then see light and brought to contribute effectively towards oncological diseases conditions which will leading to the direct, efficient and optimum identification of therapeutics and inhibitors that would then see light.

References

Ad-, V. and Alto, P. (1966) 'LYSOSOME FUNCTION IN THE REGULATION PROCESS IN CELLS GLAND OF THE SECRETORY OF THE ANTERIOR PITUITARY Techniques for Morphological Studies Downloaded from jcb . rupress . org on January 20 , 2011 Animals', *Most*.

Ahlemann, M. *et al.* (2006) 'Overexpression Facilitates mTOR-dependent Growth Transformation', *Molecular Carcinogenesis*, 967(May), pp. 957–967. doi: 10.1002/mc.

Ahmedin Jemal, DVM, P. *et al.* (1999) 'Ahmedin Jemal, DVM, PhD1 ; Freddie Bray, PhD2 ; Melissa M. Center, MPH3 ; Jacques Ferlay, ME4', *CA Cancer J Clin*, 49(2), pp. 1,33-64. doi: 10.3322/caac.20107.Available.

Aits, S. and Jäättelä, M. (2013) 'Lysosomal cell death at a glance', *Journal of Cell Science*, 126(9), pp. 1905–1912. doi: 10.1242/jcs.091181.

Akers, J. C. *et al.* (2013) 'Biogenesis of extracellular vesicles (EV): Exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies', *Journal of Neuro-Oncology*, 113(1), pp. 1–11. doi: 10.1007/s11060-013-1084-8.

Andrade, S. S. *et al.* (2016) 'Cathepsin K induces platelet dysfunction and affects cell signaling in breast cancer - molecularly distinct behavior of cathepsin K in breast

cancer', *BMC Cancer*. BMC Cancer, pp. 1–19. doi: 10.1186/s12885-016-2203-7.

Bartek, J. *et al.* (1991) 'Efficient immortalization of luminal epithelial cells from human mammary gland by introduction of simian virus 40 large tumor antigen with a recombinant retrovirus', *Proceedings of the National Academy of Sciences of the United States of America*, 88(9), pp. 3520–3524. doi: 10.1073/pnas.88.9.3520.

Bitu, C. C. *et al.* (2016) 'IL-1 α induces cathepsin K in oral cancer cells – the invasion is unaffected'.

Bonnet, N. *et al.* (2017) 'Cathepsin K Controls Cortical Bone Formation by Degrading Periostin', *Journal of Bone and Mineral Research*, 32(7), pp. 1432–1441. doi: 10.1002/jbmr.3136.

Boonen, S. *et al.* (2012) 'Inhibition of cathepsin K for treatment of osteoporosis', *Current Osteoporosis Reports*, 10(1), pp. 73–79. doi: 10.1007/s11914-011-0085-9.

Boutté, A. M. *et al.* (2011) 'Identification of a myeloid-derived suppressor cell cystatin-like protein that inhibits metastasis', *FASEB Journal*, 25(8), pp. 2626–2637. doi: 10.1096/fj.10-180604.

Breznik, B. *et al.* (2019) 'Cystatins in cancer progression: More than just cathepsin inhibitors', *Biochimie*. Société Française de Biochimie et Biologie Moléculaire (SFBBM). doi: 10.1016/j.biochi.2019.05.002.

Brix, K. *et al.* (2008) 'Cysteine cathepsins: Cellular roadmap to different functions', *Biochimie*, 90(2), pp. 194–207. doi: 10.1016/j.biochi.2007.07.024.

Brömme, D. and Lecaille, F. (2009) 'Cathepsin K inhibitors for osteoporosis and potential off-target effects', *Expert Opinion on Investigational Drugs*, 18(5), pp. 585–600. doi: 10.1517/13543780902832661.

Brömme, D. and Okamoto, K. (1995) 'Human cathepsin O2, a novel cysteine protease highly expressed in osteoclastomas and ovary molecular cloning, sequencing and tissue distribution.', *Biological chemistry Hoppe-Seyler*, 376(6), pp. 379–84. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7576232 (Accessed: 9 September 2018).

Cardoso, L. F., Maciel, L. M. Z. and de Paula, F. J. A. (2014) 'Os múltiplos efeitos das disfunções tireoidianas sobre o metabolismo osteomineral', *Arquivos Brasileiros de Endocrinologia e Metabologia*, 58(5), pp. 452–463. doi: 10.1590/0004-2730000003311.

Charhon SA, Chapuy MC, Delvin EE, Valentin-Opran A, Edouard CM, M. P. (1983) 'Histomorphometric Analysis', *Cancer*, 51(5), pp. 918–924.

Chikatsu, N. *et al.* (2000) 'Interactions between cancer and bone marrow cells induce osteoclast differentiation factor expression and osteoclast-like cell formation in vitro', *Biochemical and Biophysical Research Communications*, 267(2), pp. 632–637. doi: 10.1006/bbrc.1999.2008.

Christensen, J. and Shastri, V. P. (2015) 'Matrix - metalloproteinase - 9 is cleaved and activated by Cathepsin K', *BMC Research Notes*. BioMed Central, pp. 1–8. doi: 10.1186/s13104-015-1284-8.

Cordes, C. *et al.* (2009) 'Simultaneous expression of Cathepsins B and K in pulmonary adenocarcinomas and squamous cell carcinomas predicts poor recurrence-free and overall survival', *Lung Cancer*, 64(1), pp. 79–85. doi: 10.1016/j.lungcan.2008.07.005.

Danilin, S. *et al.* (2012) 'Myeloid-derived suppressor cells expand during breast cancer progression and promote tumor-induced bone destruction', *OncoImmunology*, 1(9), pp. 1484–1494. doi: 10.4161/onci.21990.

Doucet, A. *et al.* (2008) 'Metadegradomics: Toward in vivo quantitative degradomics of proteolytic post-translational modifications of the cancer proteome', *Molecular and Cellular Proteomics*, 7(10), pp. 1925–1951. doi: 10.1074/mcp.R800012-MCP200.

Edgington-mitchell, L. E. *et al.* (no date) 'Cysteine cathepsin activity suppresses osteoclastogenesis of myeloid-derived suppressor cells in breast cancer', 6(29), pp. 8–10.

Edwards, J. R. and Mundy, G. R. (2011) 'Advances in osteoclast biology: Old findings and new insights from mouse models', *Nature Reviews Rheumatology*. Nature Publishing Group, 7(4), pp. 235–243. doi: 10.1038/nrrheum.2011.23.

Ell, B. *et al.* (2013) 'Tumor-Induced Osteoclast miRNA Changes as Regulators and

Biomarkers of Osteolytic Bone Metastasis', *Cancer Cell*. Elsevier Inc., 24(4), pp. 542–556. doi: 10.1016/j.ccr.2013.09.008.

Everts, V. *et al.* (1992) 'Degradation of collagen in the bone-resorbing compartment underlying the osteoclast involves both cysteine-proteinases and matrix metalloproteinases', *Journal of Cellular Physiology*, 150(2), pp. 221–231. doi: 10.1002/jcp.1041500202.

Everts, V. *et al.* (2003) 'Cathepsin K Deficiency in Pycnodysostosis Results in Accumulation of Non-Digested Phagocytosed Collagen in Fibroblasts', *Calcified Tissue International*, 73(4), pp. 380–386. doi: 10.1007/s00223-002-2092-4.

Fonager, R. F. *et al.* (2017) 'Diagnostic test accuracy study of 18F-sodium fluoride PET/CT, 99mTc-labelled diphosphonate SPECT/CT, and planar bone scintigraphy for diagnosis of bone metastases in newly diagnosed, high-risk prostate cancer.', *American journal of nuclear medicine and molecular imaging*, 7(5), pp. 218–227. Available at: http://www.ncbi.nlm.nih.gov/pubmed/29181269%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5698615.

Fonović, M. and Turk, B. (2014) 'Cysteine cathepsins and extracellular matrix degradation', *Biochimica et Biophysica Acta - General Subjects*, 1840(8), pp. 2560–2570. doi: 10.1016/j.bbagen.2014.03.017.

Fradet, A. *et al.* (2011) 'Dual function of ERRα in breast cancer and bone metastasis formation: Implication of VEGF and osteoprotegerin', *Cancer Research*, 71(17), pp. 5728–5738. doi: 10.1158/0008-5472.CAN-11-1431.

Futakuchi, M., Fukamachi, K. and Suzui, M. (2015) 'Heterogeneity of tumor cells in the bone microenvironment; mechanisms and therapeutic targets for bone metastasis of prostate or breast cancer', *Advanced Drug Delivery Reviews*. Elsevier B.V. doi: 10.1016/j.addr.2015.11.017.

Gall, L. *et al.* (2007) 'A Cathepsin K Inhibitor Reduces Breast Cancer – Induced Osteolysis and Skeletal Tumor Burden', (20), pp. 9894–9903. doi: 10.1158/0008-5472.CAN-06-3940.

Gandaglia, G. *et al.* (2015) 'Impact of the Site of Metastases on Survival in Patients with Metastatic Prostate Cancer', *European Urology*, 68(2), pp. 325–334. doi: 10.1016/j.eururo.2014.07.020.

Ganly, I., Patel, S. and Shah, J. (2012) 'Early stage squamous cell cancer of the oral tongue-clinicopathologic features affecting outcome', *Cancer*, 118(1), pp. 101–111. doi: 10.1002/cncr.26229.

Gay, S., Gay, R. E. and Koopman, W. J. (1993) 'Molecular and cellular mechanisms of joint destruction in rheumatoid arthritis: Two cellular mechanisms explain joint destruction?', *Annals of the Rheumatic Diseases*, 52(SUPPL. 1). doi: 10.1136/ard.52.Suppl_1.S39.

Gupta, G. P. and Massagué, J. (2006) 'Cancer Metastasis: Building a Framework', *Cell*, 127(4), pp. 679–695. doi: 10.1016/j.cell.2006.11.001.

Hardaway, A. L., Herroon, M. K. and Rajagurubandara, E. (2015) 'Marrow adipocyte-derived CXCL1 and CXCL2 contribute to osteolysis in metastatic prostate cancer', pp. 353–368. doi: 10.1007/s10585-015-9714-5.

Hira, V. V. V. *et al.* (2015) 'CD133+ and Nestin+ Glioma Stem-Like Cells Reside Around CD31+ Arterioles in Niches that Express SDF-1α, CXCR4, Osteopontin and Cathepsin K', *Journal of Histochemistry and Cytochemistry*, 63(7), pp. 481–493. doi: 10.1369/0022155415581689.

Hol, J., Wilhelmsen, L. and Haraldsen, G. (2010) 'The murine IL-8 homologues KC, MIP-2, and LIX are found in endothelial cytoplasmic granules but not in Weibel-Palade bodies', *Journal of Leukocyte Biology*, 87(3), pp. 501–508. doi: 10.1189/jlb.0809532.

Hou, W. S. *et al.* (2003) 'Cleavage site specificity of cathepsin K toward cartilage proteoglycans and protease complex formation', *Biological Chemistry*, 384(6), pp. 891–897. doi: 10.1515/BC.2003.100.

Ishida, M., Kojima, F. and Okabe, H. (2013) 'Cathepsin K expression in basal cell carcinoma', *Journal of the European Academy of Dermatology and Venereology*, 27(1), pp. 2011–2013. doi: 10.1111/j.1468-3083.2011.04436.x.

Kenig, S. *et al.* (2011) 'Inhibition of cathepsin L lowers the apoptotic threshold of glioblastoma cells by up-regulating p53 and transcription of caspases 3 and 7', *Apoptosis*, 16(7), pp. 671–682. doi: 10.1007/s10495-011-0600-6.

Khodavirdi, A. C. *et al.* (2006) 'Increased expression of osteopontin contributes to the progression of prostate cancer', *Cancer Research*, 66(2), pp. 883–888. doi: 10.1158/0008-5472.CAN-05-2816.

Kleer, C. G. *et al.* (2008) 'Epithelial and stromal cathepsin K and CXCL14 expression in breast tumor progression', *Clinical Cancer Research*, 14(17), pp. 5357–5367. doi: 10.1158/1078-0432.CCR-08-0732.

Kolter, T. and Sandhoff, K. (2010) 'Lysosomal degradation of membrane lipids', *FEBS Letters*. Federation of European Biochemical Societies, 584(9), pp. 1700–1712. doi: 10.1016/j.febslet.2009.10.021.

Kundu, S. T. *et al.* (no date) 'TMEM106B drives lung cancer metastasis by inducing TFEB-dependent lysosome synthesis and secretion of cathepsins', *Nature Communications*. Springer US, (2018). doi: 10.1038/s41467-018-05013-x.

Le, F., Vargas, G. and Clézardin, P. (2016) 'The role of osteoclasts in breast cancer bone metastasis', *Journal of Bone Oncology*. Elsevier, pp. 1–3. doi: 10.1016/j.jbo.2016.02.008.

Lecaille, F., Bro, D. and Lalmanach, G. (2008) 'Biochemical properties and regulation of cathepsin K activity', 90, pp. 208–226. doi: 10.1016/j.biochi.2007.08.011.

Lecaille, F., Brömme, D. and Lalmanach, G. (2008) 'Biochemical properties and regulation of cathepsin K activity', *Biochimie*, 90(2), pp. 208–226. doi: 10.1016/j.biochi.2007.08.011.

Leusink, F. K. *et al.* (2018) 'Cathepsin K associates with lymph node metastasis and poor prognosis in oral squamous cell carcinoma', *BMC Cancer*. BMC Cancer, 18(1), pp. 1–9. doi: 10.1186/s12885-018-4315-8.

Li, R. *et al.* (2019) 'Gut microbiota-stimulated cathepsin K secretion mediates TLR4-dependent M2 macrophage polarization and promotes tumor metastasis in colorectal cancer', *Cell Death & Differentiation*. Springer US. doi: 10.1038/s41418-019-0312-y.

Liang, W. *et al.* (2019) 'Targeting cathepsin K diminishes prostate cancer establishment and growth in murine bone'. doi: 10.1007/s00432-019-02950-y.

Lindeman, J. H. N. *et al.* (2004) 'Cathepsin K is the principal protease in giant cell tumor of bone', *American Journal of Pathology*, 165(2), pp. 593–600. doi: 10.1016/S0002-9440(10)63323-8.

Littlewood-evans, A. J. *et al.* (1997) 'The Osteoclast-associated Protease Cathepsin K Is Expressed in Human Breast Carcinoma1', pp. 5386–5391.

López-Otín, C. and Overall, C. M. (2002) 'Protease degradomics: A new challenge for proteomics', *Nature Reviews Molecular Cell Biology*, 3(7), pp. 509–519. doi: 10.1038/nrm858.

Ludeman, M. J. *et al.* (2005) 'PAR1 cleavage and signaling in response to activated protein C and thrombin', *Journal of Biological Chemistry*, 280(13), pp. 13122–13128. doi: 10.1074/jbc.M410381200.

Mohamed, M. M. and Sloane, B. F. (2006) 'Cysteine cathepsins: Multifunctional enzymes in cancer', *Nature Reviews Cancer*, 6(10), pp. 764–775. doi: 10.1038/nrc1949.

Moore, A. (2013) 'Advances in menopausal therapy: The tissue-selective estrogen complex', *Journal of the American Academy of Nurse Practitioners*, 25(3), pp. 126–133. doi: 10.1111/j.1745-7599.2012.00804.x.

Mukherjee, K. and Chattopadhyay, N. (2016) 'Pharmacological Inhibition of Cathepsin K: a Promising Novel Approach for Postmenopausal Osteoporosis Therapy', *BIOCHEMICAL PHARMACOLOGY*. Elsevier Inc. doi: 10.1016/j.bcp.2016.04.010.

Mundy, G. R. (2002) 'Metastasis to bone: Causes, consequences and therapeutic opportunities', *Nature Reviews Cancer*, 2(8), pp. 584–593. doi: 10.1038/nrc867.

Nakano, I. (2014) 'Engulfing losers by winners in cancer: Do cancer stem cells catch eat-me signals from noncancer stem cells?', *Future Oncology*, 10(8), pp. 1335–1338. doi: 10.2217/fon.14.66.

Novinec, M. and Lenarčic, B. (2013) 'Cathepsin K: A unique collagenolytic cysteine

peptidase', *Biological Chemistry*, 394(9), pp. 1163–1179. doi: 10.1515/hsz-2013-0134.

Pišlar, A., Jewett, A. and Kos, J. (2018) 'Cysteine cathepsins: Their biological and molecular significance in cancer stem cells', *Seminars in Cancer Biology*. Elsevier Ltd. doi: 10.1016/j.semcancer.2018.07.010.

Prasmickaite, L. *et al.* (2002) 'Photochemical disruption of endocytic vesicles before delivery of drugs: A new strategy for cancer therapy', *British Journal of Cancer*, 86(4), pp. 652–657. doi: 10.1038/sj.bjc.6600138.

Puente, X. S. *et al.* (2003) 'Human and mouse proteases: A comparative genomic approach', *Nature Reviews Genetics*, 4(7), pp. 544–558. doi: 10.1038/nrg1111.

Quintanilla-Dieck, M. J. *et al.* (2009) 'Expression and regulation of cathepsin K in skin fibroblasts', *Experimental Dermatology*, 18(7), pp. 596–602. doi: 10.1111/j.1600-0625.2009.00855.x.

Raggatt, L. J. and Partridge, N. C. (2010) 'Cellular and molecular mechanisms of bone remodeling', *Journal of Biological Chemistry*, 285(33), pp. 25103–25108. doi: 10.1074/jbc.R109.041087.

Raposo, T. P. *et al.* (2015) 'Inflammation and cancer: Till death tears them apart', *Veterinary Journal*, 205(2), pp. 161–174. doi: 10.1016/j.tvjl.2015.04.015.

Ren, G. *et al.* (2012) 'Coronin 3 promotes gastric cancer metastasis via the up-regulation of MMP-9 and cathepsin K', pp. 1–10.

De Rosa, M. *et al.* (2015) 'Genetics, diagnosis and management of colorectal cancer (Review)', *Oncology Reports*, 34(3), pp. 1087–1096. doi: 10.3892/or.2015.4108.

Rosen, C. J. and Bouxsein, M. L. (2006) 'Mechanisms of disease: Is osteoporosis the obesity of bone?', *Nature Clinical Practice Rheumatology*, 2(1), pp. 35–43. doi: 10.1038/ncprheum0070.

Routy, B. *et al.* (2018) 'Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors', *Science*, 359(6371), pp. 91–97. doi: 10.1126/science.aan3706.

Saftig, P. *et al.* (1998) 'Impaired osteoclastic bone resorption leads to osteopetrosis in cathepsin-K-deficient mice', *Proceedings of the National Academy of Sciences of the United States of America*, 95(23), pp. 13453–13458. doi: 10.1073/pnas.95.23.13453.

Schmit, J. M. *et al.* (2012) 'Cathepsin K expression & activity in K9 OSA', *Journal of Veterinary Internal Medicine*, 26(1), pp. 126–134. doi: 10.1111/j.1939-1676.2011.00834.x.

Sevenich, L. *et al.* (2011) 'Transgenic expression of human cathepsin B promotes progression and metastasis of polyoma-middle-T-induced breast cancer in mice', *Oncogene*, 30(1), pp. 54–64. doi: 10.1038/onc.2010.387.

Sheridan, C. *et al.* (2006) 'CD44+/CD24-Breast cancer cells exhibit enhanced invase properties: An early step necessary for metastasis', *Breast Cancer Research*, 8(5), pp. 1–13. doi: 10.1186/bcr1610.

Siegel, R. *et al.* (2014) 'Cancer statistics, 2014', *CA: A Cancer Journal for Clinicians*, 64(1), pp. 9–29. doi: 10.3322/caac.21208.

Suoranta, S. *et al.* (2012) 'Thickened skull, scoliosis and other skeletal findings in Unverricht-Lundborg disease link cystatin B function to bone metabolism', *Bone*. Elsevier Inc., 51(6), pp. 1016–1024. doi: 10.1016/j.bone.2012.08.123.

Takayanagi, H. (2018) 'Osteoclast biology and bone resorption', *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, 4, pp. 46–53. doi: 10.1002/9781119266594.ch6.

Tamada, T. *et al.* (2001) 'Biochemical markers for the detection of bone metastasis in patients with prostate cancer: Diagnostic efficacy and the effect of hormonal therapy', *Journal of Bone and Mineral Metabolism*, 19(1), pp. 45–51. doi: 10.1007/s007740170059.

Taxel, P., Choksi, P. and Van Poznak, C. (2012) 'The management of osteoporosis in breast cancer survivors', *Maturitas*. Elsevier Ireland Ltd, 73(4), pp. 275–279. doi: 10.1016/j.maturitas.2012.08.009.

True, L. D. *et al.* (2003) 'Cathepsin K mRNA and Protein Expression in Prostate Cancer

Progression*', 18(2), pp. 222–230.

Tumminello, F. M. *et al.* (2008) 'Circulating cathepsin K and cystatin C in patients with cancer related bone disease: Clinical and therapeutic implications', *Biomedicine and Pharmacotherapy*, 62(2), pp. 130–135. doi: 10.1016/j.biopha.2007.07.001.

Turk, V., Stoka, V., Vasiljeva, O., Renko, M., Sun, T. and Turk, B. (2012) 'Biochimica et Biophysica Acta Cysteine cathepsins : From structure , function and regulation to new frontiers ☆', 1824, pp. 68–88. doi: 10.1016/j.bbapap.2011.10.002.

Turk, V., Stoka, V., Vasiljeva, O., Renko, M., Sun, T., Turk, B., *et al.* (2012) 'Cysteine cathepsins: From structure, function and regulation to new frontiers', *Biochimica et Biophysica Acta - Proteins and Proteomics*, 1824(1), pp. 68–88. doi: 10.1016/j.bbapap.2011.10.002.

Valastyan, S. and Weinberg, R. A. (2011) 'Tumor metastasis: Molecular insights and evolving paradigms', *Cell*. Elsevier Inc., 147(2), pp. 275–292. doi: 10.1016/j.cell.2011.09.024.

Vasiljeva, O. *et al.* (2006) 'Tumor cell-derived and macrophage-derived cathepsin B promotes progression and lung metastasis of mammary cancer', *Cancer Research*, 66(10), pp. 5242–5250. doi: 10.1158/0008-5472.CAN-05-4463.

Verbovšek, U. *et al.* (2014) 'Expression analysis of all protease genes reveals cathepsin K to be overexpressed in glioblastoma', *PLoS ONE*, 9(10). doi: 10.1371/journal.pone.0111819.

Vidak, E. *et al.* (2019) 'Cysteine Cathepsins and their Extracellular Roles: Shaping the Microenvironment', *Cells*, 8(3), p. 264. doi: 10.3390/cells8030264.

Westhoff, M. A. *et al.* (2013) 'Inhibition of NF-κB signaling ablates the invasive phenotype of glioblastoma', *Molecular Cancer Research*, 11(12), pp. 1611–1623. doi: 10.1158/1541-7786.MCR-13-0435-T.

Xue, M. *et al.* (2014) 'Endogenous MMP-9 and not MMP-2 promotes rheumatoid synovial fibroblast survival, inflammation and cartilage degradation', *Rheumatology (United Kingdom)*, 53(12), pp. 2270–2279. doi: 10.1093/rheumatology/keu254.

 doi:10.20944/preprints202003.0405.v1

Yan, X. *et al.* (2011) 'Stromal expression of cathepsin K in squamous cell carcinoma', *Journal of the European Academy of Dermatology and Venereology*, 25(3), pp. 362–365. doi: 10.1111/j.1468-3083.2010.03743.x.