Toxic effects of some heavy metals on the structure and stability of the recombinant human FGFR2b kinase domain

Running title: The effect of toxic heavy metals on FGFR2b

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Abstract

Fibroblast growth factor receptor type II (FGFR2b) is crucial in mediating cellular signal transduction and controlling vital biological processes such as cell growth and differentiation. The disruption or impairment of signaling pathways mediated by this particular receptor has

been closely associated with the pathophysiology of various human malignancies, including breast cancer, ovarian cancer, and endometrial cancer. It has been observed that toxic heavy metals, such as Lead, Cadmium, and Aluminum, exert their detrimental effects primarily through the alteration of established signaling pathways within the cellular environment. The primary objective of this research endeavor is to conduct a comprehensive investigation into the effects of the heavy metals Lead (Pb^{2+}), Cadmium (Cd^{2+}), and Aluminum (Al^{3+}) on both the structural integrity and stability of recombinant FGFR2b. The analysis of intrinsic fluorescence emission and circular dichroism (CD) spectra of FGFR2b, when exposed to varying concentrations of these heavy toxic metals, indicated a gradual series of structural fluctuations that corresponded with the increased concentrations of the metals present. Furthermore, the findings from fluorescence and Fourier-transform infrared (FTIR) analysis of the protein structure demonstrated that the influence exerted by Pb^{2+} at concentrations of 100 and 500 µM was significantly more pronounced and impactful than the effects produced by the other two metals under investigation The structure and stability of FGFR2b as a key receptor in cellular signal transduction were reduced by Pb^{2+}. These results shed light on the effect of toxic heavy metals on biological functions of the cells via a change in their signaling pathways.

Keywords: Fibroblast growth factor receptor, Kinase domain, Purification, Structure, Stability.

1. Introduction

Fibroblast growth factors (FGF) and their receptors (FGFR) play crucial roles such as cell proliferation, differentiation, migration, and survival. They are essential to fetal growth, development, and regulation of angiogenesis and wound healing in adults (1). The mammalian fibroblast growth factors (FGFs) are involved in critical signaling pathways, and their dysregulation can result in anomalies (2). The pleiotropic effects of FGFs are interposed through four highly conserved receptor tyrosine kinases (RTK), namely FGF receptors 1 to 4 (FGFR1–FGFR4). The structure of FGFRs consists of three extracellular immunoglobulin (D1-D3) domains, the membrane, and intracellular tyrosine kinase activity portions. The FGF/FGFR pathways significantly influence various cell behaviors and are implicated in numerous human diseases, like cancer (3). FGFR2, through gene amplification and abnormal

activity, is a therapeutic target in cancers (4). GFR2 has two isoforms, FGFR2b and FGFR2c, and its locus is located at 10q26.13. FGFR2b comprises 334 aa and has a molecular weight of 38 aa (5). The change of FGFR2 performance by different factors such as somatic missense mutations or gene amplification relays in a variety of cancers including endometrial, lung, breast, gastric, colorectal, and ovarian cancers (6).

Heavy metals, originating from industrial and agricultural sources, are major environmental pollutants. Their significant toxicity, even at low exposure levels, poses substantial risks to biological systems by disrupting signaling pathways and inducing DNA damage, oxidative stress, and structural mutations (6, 7). The toxicological traits of Pb^{2+} and Cd^{2+} are partly accompanied by the generation of reactive oxygen species (ROS) that may incur numerous intracellular signaling pathways, especially NF-kB, JNK/SAPK/p38, as well as ERK/MAPK (7). The induction of the transcription nuclear factor κB (NF-κB) by metal-triggered signaling routes may result in sustained inflammatory processes and associated conditions, such as cancer development. Research findings have illustrated that cadmium notably triggers NF-κB and the secretion of the chemokine IL-8 (8).

Despite the known roles of FGFR2b in cellular signaling and the toxicity of heavy metals in disrupting these pathways, little is known about how these metals affect FGFR2b structure. This study aims to evaluate the effects of Lead, Cadmium, and Aluminum on FGFR2b using CD, fluorescence, and FTIR spectroscopy.

2. Materials & Methods

In this study, imidazole, IPTG and Ni^{2+} -NTA from Sigma (Sigma-Aldrich Co), ampicillin and HEPES from Merck and E.coli bacteria BL21 (DE3) were obtained from the company invitrogen. All other reagents used in this study were of analytical grade and purchased from Sigma-Aldrich Co.

2.1. Expression and purification of FGFR2b kinase domain

Recombinant pLEICS-01 vectors containing the mutated coding region of FGFR2b were transformed into the standard E. coli BL21 (DE3) host cells. Luria- Bertani broth and LB agar bacteria culture media in the presence of 100 µg/ml ampicillin were inoculated with antibiotics. Susceptible cells were prepared in the presence of calcium chloride.

Recombinant pLEICS-01 vectors containing the target gene were transformed into E. coli BL21 86 (DE3). The expression of recombinant protein was induced by 1mM IPTG. The expressed protein was largely insoluble and prone to aggregation. Protein solubility was performed by changing in temperature condition of the bacterial culture from 37ºC to 20ºC. The purified protein was obtained using Ni^{2+} 90 -NTA column by affinity chromatography procedure and eluting buffer containing 50-200 mM imidazole. The fractions containing FGFR2b kinase domain were dialyzed in 25 mM Tris-HCl, 100 mM NaCl, PH 7.5, subdivided into smaller aliquots and stored at -80 c. Functional analysis of the purified kinase domain was performed by polyacrylamide gel electrophoresis (PAGE). The concentration of protein was measured with Nano Drop spectrophotometry with an extinction coefficient at 280 nm of 41160 M^{-1} cm^{-1}.

The engineered kinase domain of human FGFR2b were considered with two mutations and cloned in our previous study (9). The mutations were created in E565A and K660E residues, selected based on associated pathologic disorders of Pfeiffer syndrome and endometrial cancer, respectively. To prove that the purified protein is in the active state, its interaction with both the wild type and mutant SH2 domains of phospholipase C (PLC) was studied using PAGE (10).

2.2. Tertiary structure of FGFR2b by internal fluorescence spectroscopy

Intrinsic fluorescence measurements of purified proteins were carried out on a Cary Eclipse spectrofluorimeter (Varian, Australia), using a 10-mm course duration quartz cuvette. Photomultiplier tube (PMT) detector was set to medium voltage. Both excitation and emission slits were adjusted with a 5 nm band pass. The internal fluorescence spectra were obtained excitation of protein (tryptophan fluorphore) at 280 nm and the emission range of 300–450 nm at room temperature. For taking the clue of record severity of the buffer, the system was zeroed with a blank dilution (400 µL of buffer). The protein concentration of 3 mg/ml was kept fixed in each sample solutions test. All the spectra gathered in the fixed concentration of EGFR2b and gradually increment in the concentrations of Pb^{2+} (100-1000 µM), Cd^{2+} (100-1000 µM) and Al^{3+} (100-1000 µM) and 3 min incubation time for each increment.

2.3. Fluorescence spectroscopy and protein chemical denaturation

The change in the λmax of emission (blue and red shift) of the folded native FGFR2b protein was 115 obtained by titration of guanidine hydrochloride (GnHCl) condensations (0-6 M).

The excitation 116 wavelength was set at 280 nm and the emission spectra were recorded in the range of 300-450 nm. FGFR2b KD Chemical denaturation and λmax of emission were achieved in the GnHCl condensations of 0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5 and 6 M, and in the different concentrations of Pb^{2+} 120 (0, 100, 150, 200,300 and 500 µM), Cd^{2+} (0, 6 and 12mM) and Al^{3+} (0, 0.5 and 1M).

2.4. Secondary structure of FGFR2b by Far UV CD spectroscopy

The Far-UV CD spectra were used to the assessment of secondary structural changes in of FGFR2b KD after incubation by different concentrations of GnHCl (0, 3, 6 M) using Jasco J-810 spectropolarimeter. Far-UV CD spectra were recorded at 25 °C from 190 to 260 nm using a quartz cuvette with 1-mm track length containing 15µM concentration of protein. CD spectra were base lined for the buffer and then the sample spectra were obtained from sole protein and its incubation by Pb^{2+} 129 (500 µM), Cd^{2+} (12 mM) and Al^{3+} (1 M). These concentrations induced different fluctuations of protein structure by fluorescence technique.

2.5. Fourier transform infrared spectroscopy of FGFR2b

The FTIR spectroscopy was used to evaluate the type of metal interaction with FGFR2b KD using a Perkin-Elmer Spectrum RXI (Infrared spectroscopy). FTIR spectra fixed concentration of sole protein and its incubation with two concentrations of Pb^{2+} 300 and 500 µM was determined.

3. Results

3.1. Expression and purification of FGFR2b kinase domain region

From SDS-PAGE analysis illustrated in Figure 1 the maximum expression of recombinant protein achieved in three and four hours post induction. There was no contamination of other bacterial proteins that may co-eluted with the purified protein.

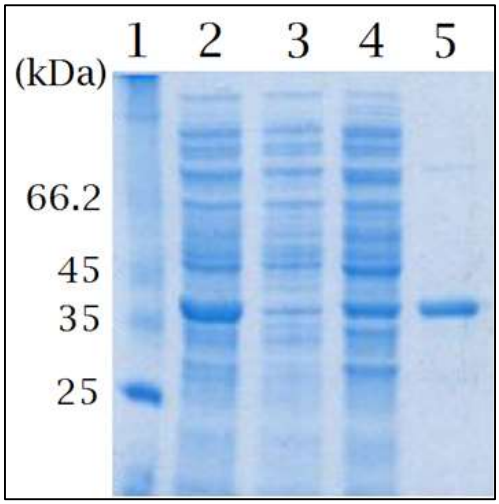

Figure 1: Purification of FGFR2b KD by affinity chromatography. Lane 1: molecular weight of markers, Lanes 2: load samples, lanes 3: flow through, and lanes 4-5: eluted samples.

3.2. Internal fluorescence analysis of FGFR2b KD

Native versus denatured spectra

Two different spectrums of internal fluorescence of tryptophan fluorphore in figure 2 are refer to the denatured and native forms of FGFR2b. The maximum emission wavelength (λmax) of the native protein in 336 nm demonstrates a red-shift toward 365 nm after incubation with 1M GnHCl as a chemical denaturant.

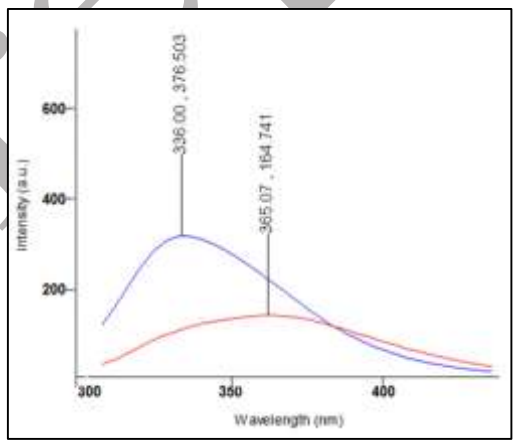

Figure 2: Internal fluorescence spectrua of native (linear line) FGFR2b KD and incubation with 1M 154 GnHCl as a chemical denaturant (dash line).

3.3. Metal increments spectra of protein

The effect of the different concentrations of Pb^{2+}, Cd^{2+} and Al^{3+} from 100µM to 1000µM were done on the fluorescence emission spectra of native FGFR2b kinase domain. As it can

observe in Figure 3 there wasn't a significant changed in the emission maximum wavelength of protein by gradually increasing of chemical concentrations while a gradually decreasing in the fluorescence intensity could be observed.

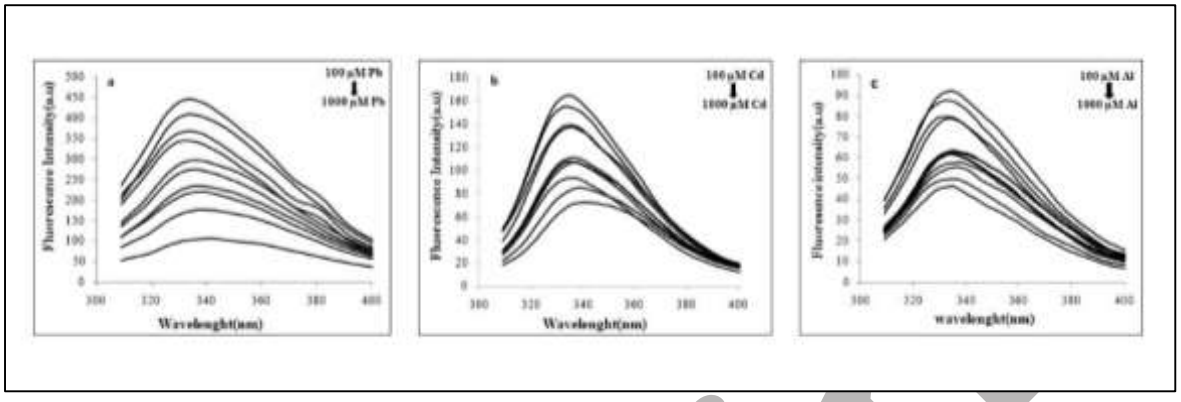

Figure 3: Fluorescence spectrum for KD of FGFR2b. (a) Represents fluorescence emission spectra at different concentrations of Pb^{2+}, (b) Cd^{2+} and (c) Al^{3+}.

3.4. Protein chemical denaturation and shift in the maximum emission spectra

As mentioned above the protein concentration was constant in all experiments and the effect of these metals on the structural changes of the protein has been down on different concentrations. The tertiary structure of the protein tyrosine kinase FGFR2b was assessed in the presence of Pb^{2+} (0, 100, 150, 200, 300 and 500 µM), Cd^{2+} (0, 6 and 12 mM) and Al^{3+} (0, 0.5 and 1 M) using intrinsic fluorescence and the change in the λmax of emission in the addition of GnHcl to the protein. As shown in Figure 4a-c the fluorescence emission spectra of protein was accompanied by a gradual decrease in intensity and red shift change of spectra.

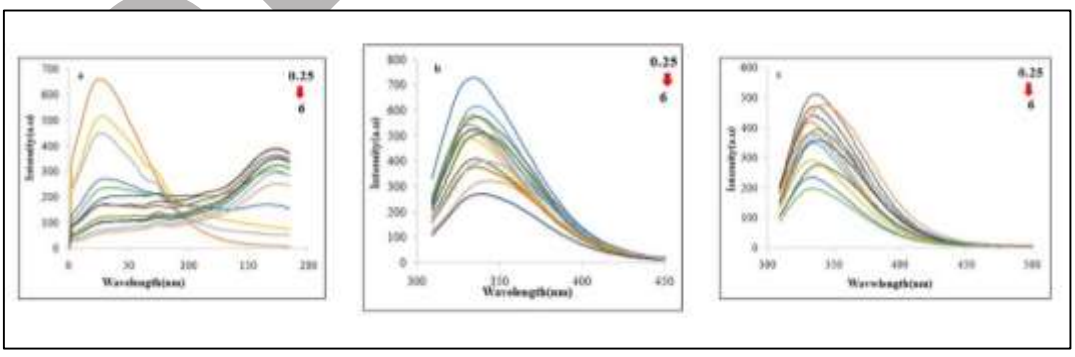

Figure 4: Internal fluorescence spectra of the FGFR2b by gradually addition of GnHcl (0.25-6M) as chemical denaturant in the presence of Pb^{2+} 500µM (a), Cd^{2+} 12 mM (b) and Al^{3+} 1M(c).

As depicted in Figure 5a-c titration of GnHcl in the presence of metals induced an observable shift in the λmax in the range of 334-340nm. The effects of chemical denaturation and change

of maximum emission intensity by gradual increment of guanidine hydrochloride in the presence of the maximum concentration of these metals illustrated in Figure 5 proved the significant change of the tertiary structure of the protein by Pb^{2+} in comparison with Cd^{2+} and Al^{3+}.

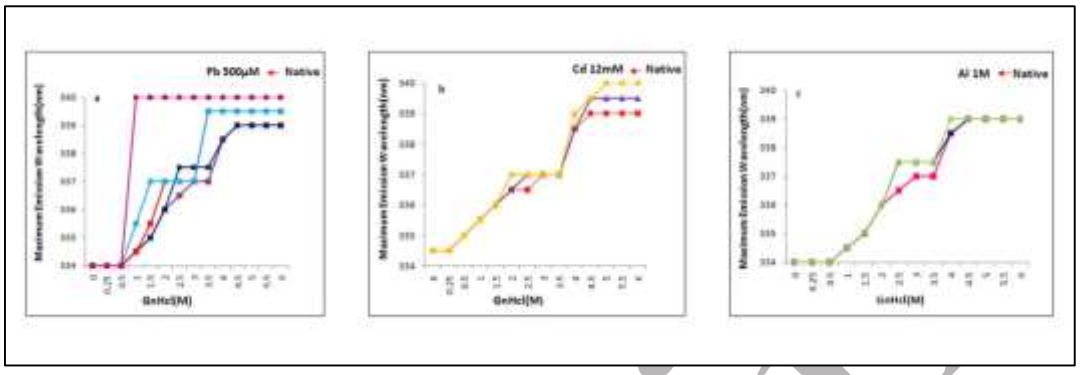

Figure 5: Red shift (334-340nm) of maximum emission intensity of FGFR2b by gradually addition of GnHcl (0.25-6M) as chemical denaturant in the presence of Pb^{2+} (0, 100, 150, 200, 300 and 500 µM) (a) Cd^{2+} (0, 6 and 12mM) (b) and Al^{3+} (0, 0.5 and 1M) (c).

3.5. Far UV circular dichroism spectroscopy and FTIR analysis for FGFR2b KD

The Far-UV CD spectra of FGFR2b kinase domain shown in Figure 6a-c using GnHcl in the concentration of 0,3 and 6 M respectively, and the presence of Cd^{2+} (0, 6 mM) and Al^{3+} (0, 0.5 M). It is clear from these figures that the effects GnHcl as a chemical denaturant changed the 193 normal shape of protein CD spectra with the increasing concentration, but this metal didn't show 194 8 a significant change inspectra. Although the Pb^{2+} treatment completely abolishes the negative elipticity of the protein.

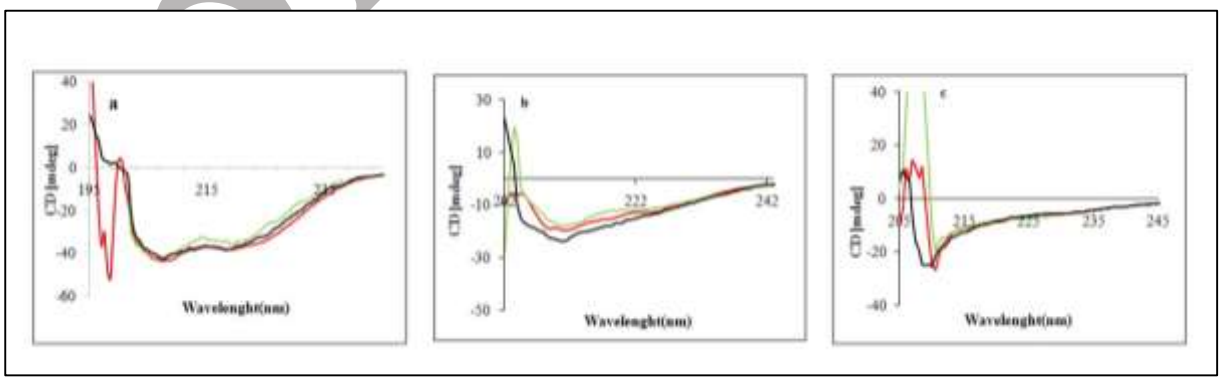

Figure 6: Spectrum Far-UV CD spectra of FGFR2b in the presence of Cd^{2+} (0, and 6mM) and Al^{3+} (0, 0.5 M) (c) by its incubation of GnHcl 0, 3M (a), 3M (b) and 6M (c) as chemical denaturant.

The significant change of Pb^{2+} on protein structure leads us to do an FTIR test for the detection of its interaction with FGFR2b Kinase Domain. As illustrated in the representation provided in figure 7, which also corresponds to the third methodology employed in this study, the Fourier Transform Infrared Spectroscopy (FTIR) analysis has corroborated and confirmed the substantial influence exerted by lead ions (Pb^{2+}) on the structural composition and characteristics as evidenced in the visual depiction presented in (Figure 7).

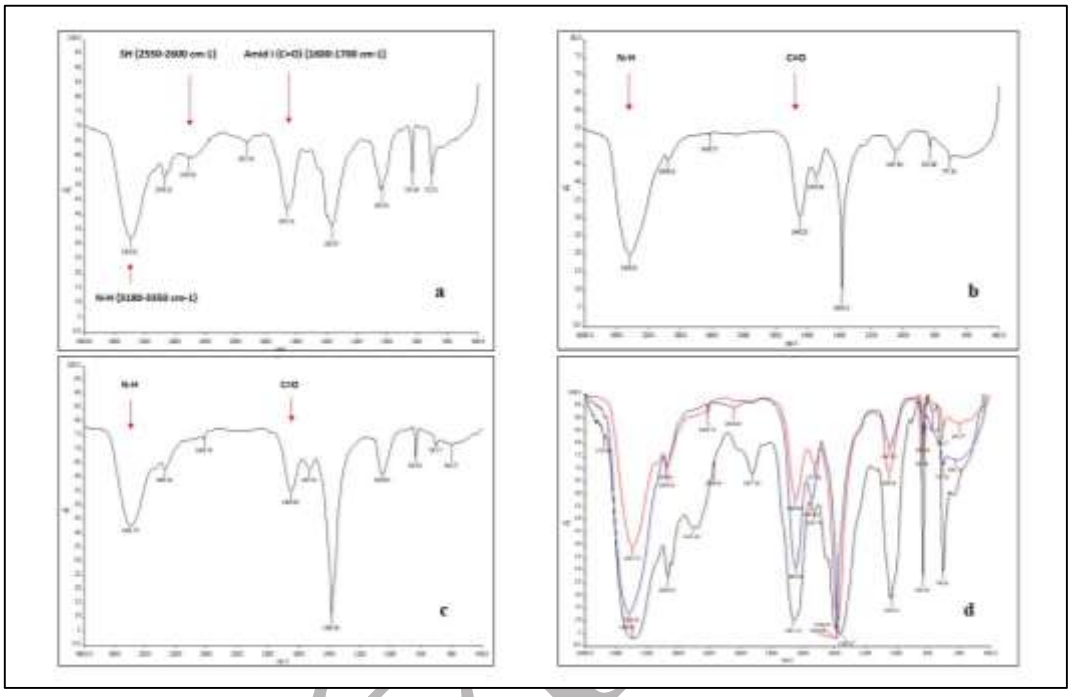

Figure 7: FTIR spectra of FGFR2b in the presence of different concentration of Pb^{2+} (0µM) (a), 208 (300µM) (b) and (500µM) (c).

4. Discussion

Given the fact that FGF signaling plays a crucial role in the regulation of several biological processes including cellular differentiation and proliferation, it is reasonable to speculate that metal toxicity can affect this signaling pathway by interaction with the related receptors. Liang Xie et al. investigated FGFR2 amplification in gastric cancer and the therapeutic potential of AZD4547, a potent ATP competitive receptor tyrosine kinase inhibitor of fibroblast growth factor receptor (FGFR) 1–3, in patients with FGFR2-amplified gastric cancer. The study showed that AZD4547 effectively blocked the phosphorylation of FGFR2 and its downstream signaling molecules, leading to apoptosis in SNU-16 cells (11).

Despite their different properties, metal carcinogens share common cancer-causing mechanisms like oxidative stress, blocking DNA repair, activating growth signals, and altering gene expression. These effects are mostly due to protein interactions, which are more important than direct DNA damage, impacting DNA repair, tumor suppression, and signaling proteins (12). This study aims to evaluate metal interactions and protein structural changes using three techniques: fluorescence, CD, and FTIR spectroscopies. Fluorescence spectroscopy is a sensitive method for characterizing protein structure, particularly through the intrinsic fluorescence of tryptophan residues used to label proteins (13). Fourier transform infrared spectroscopy is one of the oldest and well established experimental techniques for the analysis of chemical bases and secondary structure of polypeptides and proteins (14). In addition, circular dichroism (CD) is recognized as a valuable technique for examining the regular structure of proteins in solution and presents important information about the secondary structure of biological macromolecules (15). The analysis of CD spectra can therefore yield valuable information about the secondary structure of biological macromolecules.

These techniques were used to study the effects of heavy metals like lead, cadmium, and aluminum on the FGFR2b kinase domain (16). In this way, different concentrations of metals with a fixed concentration of protein were investigated on the structure and stability of protein. The amount of heavy metals in the human body is often found in biological fluids like blood, plasma, and urine. These metals are derived from workplaces or situations where people are exposed to them, leading to damage to cells and important proteins (17).

These heavy metals and their complexes can induce unwilling biological effects, e.g. Pb^{2+} can inhibit enzyme activity with coordination by sulfhydryl groups (SH). The clearest manifestation 247 of this interaction impairment of the biosynthesis of porphyrin metabolism in humans that associated with the disorder (18). The test of FGFR2b KD activity was approved by its interaction with both the wild-type and mutant SH2 domains of phospholipase C (PLC). So, in this case, the complexation of Pb^{2+} with this substrate can induce its toxic effects or cover as a metabolite interposition in a signaling pathway (10, 16).

As it can observe from Figure 5a-c the chemical denaturation of FGFR2b KD obeyed from a three-stage process. This type of denaturation emphasizes the cooperativity of unfolding and two sigmoidal shapes of denaturation may be related to the two domains of the protein. As shown in Figure 5c the Pb^{2+} in its higher toxic concentration induced a quick co-destruction

of two domains in protein structure and reduced its stability without transition from intermediate state. In a study conducted by P. Apostoli et al, the effects of Pb^{2+} on anion channel membrane proteins of RBC, was approved. In that case, the concentration of Pb^{2+} in 60.6 micrograms/100 ml of worker's blood test showed a significant decrease in the band of channels that appeared on the SDS- PAGE (19).

Various studies have examined the toxic effects of Pb^{2+} on the cellular and molecular levels, especially in the signaling pathways of the neurons. It has been shown that Pb^{2+} can aggregate in the brain when its concentrations in the blood increase. The astroglia is the most liable cell type for Pb^{2+} accumulation and stockpile in the central nervous system (CNS) (20). One potential mechanism implicated in the toxicity or poisoning caused by Pb^{2+} is its capacity to trigger oxidative stress in blood and various tissues, thus contributing to the development of poisoning through disruption of the intricate balance between pro oxidants and antioxidants present in mammalian cells. Numerous researchers have proposed a potential role of reactive oxygen species (ROS) in Pb-induced toxicity, whereby Pb leads to elevated levels of the lipid peroxidation marker malondialdehyde (MDA) and reduces the activities of key antioxidant enzymes such as glutathione peroxidase (GPx) and superoxide dismutase (SOD) in the brains of rats (21).

ERK1/2, known as extracellular signal-regulated kinase 1/2, plays a crucial role in forming long-term potentiation (LTP), and the presence of Pb^{2+} can disrupt LTP by interfering with ERK and other signal molecules. Basic fibroblast growth factor (bFGF) is a neurotrophic factor that helps protect neural cells from toxins, potentially through the MEK1/ERK signal pathway (20). Besides, chronic exposure to Al significantly diminishes the activities of protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) and reduces the expression levels of extracellular signal-regulated kinases (ERK1/2) (22). Additionally, Al^{3+} can impact the expression and processing of amyloid precursor protein receptor in Alzheimer's disease. Researchers have observed that Al(mal)3 affects PKC enzyme activity and NMDAR expression through mGluR1, pointing to aluminum's role in synaptic plasticity and providing insights into complex neurotoxic mechanisms (23). Also, Cd^{2+} can crack cellular PKC which ultimately gains phosphorylation of various transcription factors which in turn leads to activation of target gene expression (24). Also Bimonte and his colleagues demonstrate that Cd^{2+} can modify the expression and pattern of estrogen receptor and androgen receptor in breast cancer cell lines and potentially leading to a carcinogenic microenvironment (25).

The use of infrared spectroscopy allows the elimination of protein conformation in a diverse range of environments. As shown in the Figure 7 the specific absorption bands of protein infrared spectra between 400 and 4000 cm $^{-1}$ in the concentrations of 300 and 500 μM Pb^{2+} induced a significant change in the shape, position and intensity of FTIR bands in comparison with the sole protein. The peak of native protein at 1527.4 cm^{-1} assigned as random coil shifted to 1546.6 and 1533.7 cm^{-1} and increased largely in the concentrations of 300 and 500 μM Pb^{2+}.

Although the magnitudes of anti-parallel β-sheet (1286.2 cm^{-1} shifted here at 1384.2 cm^{-1}) had not changed in native and Pb^{2+} incubated protein, the peak at 1649.8 cm^{-1} (shifted to 1648.2 and 1661.1 cm^{-1}) which assigned as α-helix and β-turn increased largely after incubation of protein with Pb^{2+}. So, this trend can be interpreted in terms of an increase in β-turn structure at the expense of α-helical structure in the presence of Pb^{2+}.

5. Conclusion

This study investigates the impact of heavy metals, including lead (Pb^{2+}), cadmium (Cd^{2+}), and aluminum (Al^{3+}), on the structure and stability of the FGFR2b kinase domain. The results show that these metals can alter the protein's structure and reduce its stability. Specifically, Pb^{2+} at higher concentrations leads to the rapid degradation of both protein domains, decreasing its stability without transitioning through an intermediate state. These findings highlight the toxic effects of heavy metals on protein structure and their potential influence on biological processes such as cancer and neurological diseases. However, the study is limited to the structural level of the protein, and the effects of these metals on cellular function and metabolic processes have not been fully explored. Further research is needed to better understand these effects in real biological systems.

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Authors' Contribution: NG* conceived of the study; MT conducted the experiments; DI, HP and MS 304 analyzed the data; MT, RK and NG wrote the manuscript; NG* and HP, DI, MS, MT, RK, SGK and NG reviewed and edited the manuscript; All authors read and approved the final version.

Ethics: We have not used any animals in this study to obtain ethical approval and we have used standardized protocols and procedures.

Conflict of Interest: The authors declare that there is no conflict of interest that could be perceived as prejudicial to the impartiality of the reported research.

Data Availability: The data that support the findings of this study are available on request from the corresponding author.

8. References

1. Xie Y, Su N, Yang J, Tan Q, Huang S, Jin M, et al. FGF/FGFR signaling in health and disease. Signal transduction and targeted therapy. 2020;5(1):181.
2. Ho BB, Bergwitz C. FGF23 signalling and physiology. Journal of molecular endocrinology. 2021;66(2):R23-r32.
3. Matsuda Y, Ueda J, Ishiwata T. Fibroblast growth factor receptor 2: expression, roles, and potential as a novel molecular target for colorectal cancer. Pathology research international. 2012;2012.
4. Nguyen AL, Facey COB, Boman BM. The Complexity and Significance of Fibroblast Growth Factor (FGF) Signaling for FGF-Targeted Cancer Therapies. Cancers. 2024;17(1).
5. Moghadasi M, Ilghari D, Gheibi N, Sirati-Sabet M, Khabbaz F, Piri H. Expression, purification and evaluation of the recombinant human fibroblast growth factor receptor 2b kinase domain. Journal of Inflammatory Diseases. 2014;18(2):4-11.
6. Katoh M. Cancer genomics and genetics of FGFR2. International journal of oncology. 2008;33(2):233-7.
7. Henkler F, Brinkmann J, Luch A. The role of oxidative stress in carcinogenesis induced by metals and xenobiotics. Cancers. 2010;2(2):376-96.
8. Freitas M, Fernandes E. Zinc, cadmium and nickel increase the activation of NF-κB and the release of cytokines from THP-1 monocytic cells. Metallomics. 2011;3(11):1238-43.
9. Kalinina J, Dutta K, Ilghari D, Beenken A, Goetz R, Eliseenkova AV, et al. The alternatively spliced acid box region plays a key role in FGF receptor autoinhibition. Structure. 2012;20(1):77-88.
10. Bae JH, Lew ED, Yuzawa S, Tomé F, Lax I, Schlessinger J. The selectivity of receptor tyrosine kinase signaling is controlled by a secondary SH2 domain binding site. Cell. 2009;138(3):514-24.
11. Xie L, Su X, Zhang L, Yin X, Tang L, Zhang X, et al. FGFR2 gene amplification in gastric cancer predicts sensitivity to the selective FGFR inhibitor AZD4547. Clinical cancer research. 2013;19(9):2572-83.
12. Beyersmann D, Hartwig A. Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Archives of toxicology. 2008;82:493-512.
13. Harding SE, Chowdhry BZ. Protein-ligand Interactions: Hydrodynamics and Calorimetry; a Practical Approach: Oxford University Press; 2001.
14. Kong J, Yu S. Fourier transform infrared spectroscopic analysis of protein secondary structures. Acta biochimica et biophysica Sinica. 2007;39(8):549-59.
15. Kelly SM, Jess TJ, Price NC. How to study proteins by circular dichroism. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics. 2005;1751(2):119-39.
16. Sridhar N, Senthilkumar J, Subburayan M. Removal of Toxic Metals (Lead & Copper) From Automotive Industry Waste Water By Using Fruit Peels. Int J Adv Inf Commun Technol. 2014;1:188-91.

17. Kasperczyk S, Słowińska-Łożyńska L, Kasperczyk A, Wielkoszyński T, Birkner E. The effect of occupational lead exposure on lipid peroxidation, protein carbonylation, and plasma viscosity. Toxicology and industrial health. 2015;31(12):1165-71.
18. Haq R, Khan MF, Haq E. Heavy Protein Alteration under the Effects of Lead Acetate in Bactrocera cucurbitae. Journal of Basic & Applied Sciences. 2012;8(2).
19. Apostoli P, Romeo L, De Matteis M, Menegazzi M, Faggionato G, Vettore L. Effects of lead on red blood cell membrane proteins. International archives of occupational and environmental health. 1988;61:71-5.
20. Zhang Y, Ye L-p, Wang B, Cao S-c, Sun L-g. Effect of lead on ERK activity and the protective function of bFGF in rat primary culture astroglia. Journal of Zhejiang University SCIENCE B. 2007;8:422-7.
21. Nnachi IS, Finbarrs-Bello E, Ozor II, Mba CE, Ugwu V. Protective Roles of Quercetin, Vitamin C and Pyridoxine on Lead Neurotoxicity via Enhanced Haematopoietic and Antioxidants Components. GLOBAL ONLINE JOURNAL OF ACADEMIC RESEARCH (GOJAR). 2023;2(1).
22. Wang B, Xing W, Zhao Y, Deng X. Effects of chronic aluminum exposure on memory through multiple signal transduction pathways. Environmental Toxicology and Pharmacology. 2010;29(3):308-13.
23. He C, Zhao X, Li H, Wang F, Zhang J, Wang Y, et al. Regulation of mGluR1 on the expression of PKC and NMDAR in aluminum-exposed PC12 cells. Neurotoxicity Research. 2021;39:634-44.
24. Checconi P, Sgarbanti R, Celestino I, Limongi D, Amatore D, Iuvara A, et al. The environmental pollutant cadmium promotes influenza virus replication in MDCK cells by altering their redox state. International Journal of Molecular Sciences. 2013;14(2):4148-62.
25. Bimonte VM, Catanzaro G, Po A, Trocchianesi S, Besharat ZM, Spinello Z, et al. The endocrine disruptor cadmium modulates the androgen–estrogen receptors ratio and induces inflammatory cytokines in luminal (A) cell models of breast cancer. Endocrine. 2024;83(3):798-809.